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THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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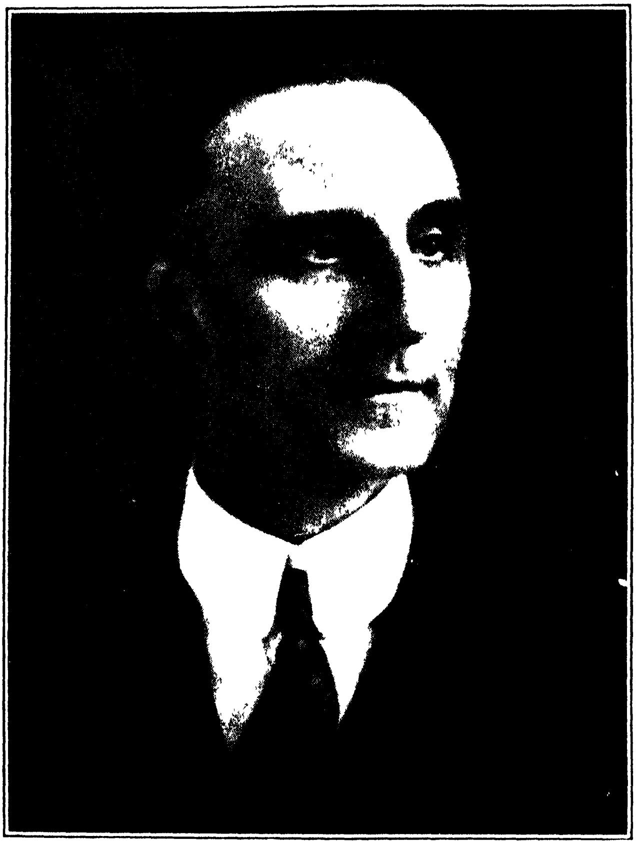

ROSCOE EDWARD DOOLITTLE, 1874—1926

ROSCOE EDWARD DOOLITTLE

Of the many serious losses which our society has suffered in the past few years none has brought so great a sense of bereavement and regret as the recent passing of R. E. Doolittle, Chief of the Central District of the Bureau of Chemistry. In his death the United States Department of Agriculture has lost one of its most loyal members and the Association of Official Agricultural Chemists one of its most active workers. While Mr. Doolittle had not been in the best of health for a number of years, no serious apprehension had been entertained regarding his physical condition. He worked at the accustomed duties of his Chicago office the morning of Friday, April 23, but during that afternoon he was stricken at his desk, where he was later found in an unconscious condition. Notwithstanding the best assistance which wifely devotion and medical skill could render he failed to rally from the stroke and passed peacefully away at his home in Evanston, Illinois, at 1:45 P. M. on Sunday, April 25, 1926.

Roscoe Edward Doolittle was born at Fowlerville, Michigan, on January 16, 1874. After attending the high schools of Howell and Morrice in his native state he entered the Michigan State College of Agriculture and Applied Science in East Lansing, where he obtained the degree of B.S. in 1896. All who knew him at college speak of his energy and industry,—the same traits which he displayed so constantly throughout his subsequent career. Immediately after graduation young Doolittle took a short course in food analysis under Prof. A. B. Prescott at the University of Michigan and then accepted his first position as assistant chemist of the Michigan State Dairy and Food Department in Lansing. In 1899 he was promoted to the position of State Chemist and continued in this office for the next five years. During this connection, in 1900, in collaboration with W. H. Hess, he published his first contributions to the subject of food analysis in papers relating to methods for the detection of "process" or "renovated" butter and to the solids and ash of cider vinegar. On September 1, 1904, he resigned his position as State Chemist of Michigan to enter the service of the United States Bureau of Chemistry as food and drug inspection chemist with headquarters at New York, where he had been delegated to enforce the Importation Act of 1904. From the small room which he fitted up for this purpose in a corner of the tenth story of the Appraiser's Stores the present spacious laboratories of the Bureau of Chemistry's New York Station have gradually evolved.

Mr. Doolittle's extensive preliminary experience as a food control official in State work at Lansing and in Federal import work at New York made him one of the best qualified men in the United States to promote the enforcement of the Federal Food and Drugs Act after this measure was passed in 1906. His contributions to the service at this critical stage were of a most constructive character, and he became recognized as a leader in this new field of regulatory control. In October, 1911, he became a member of the Board of Food and Drug Inspection and, after the resignation of Dr. Wiley, was made acting chief of the Bureau of Chemistry, serving in this capacity from March 15 to December 15, 1912. He discharged the duties of that arduous position during a trying transition period in a most commendable manner.

On January 1, 1917, after the initiation of the present district system, Mr. Doolittle was made chief of the Eastern District of the Bureau of Chemistry, but at the end of that year he was transferred to Chicago, where he occupied the position of chief of the Central District until the time of his death.

It is impossible in this short review to do adequate justice to the splendid ability and self-sacrificing labors of Mr. Doolittle during the twenty-five years of his connection with the Association of Official Agricultural Chemists. No chemist was more ready than he to assume the responsibilities which were placed upon him, and during the forty-two years of the association's history no member has filled so many of its different offices. His first attendance was at the eighteenth annual convention in November, 1901, while he was State Chemist of Michigan; at this meeting the Doolittle-Hess method for detecting renovated butter was first discussed by the Referee on Dairy Products. In 1902 he was appointed Associate Referee on Spices, on which subject he read a report at the 1903 meeting[1]. His collaborative work upon oils and his method for detecting azo dyes and annatto in butter were also reported upon at this convention[2]. Very pertinent also to the work of the association at this time were his remarks upon flavoring extracts[3], upon which subject he was appointed associate referee. He did collaborative work upon lard tests[4] for the 1905 meeting, and in 1906 he read a report as Associate Referee on Condiments[5]. For the 1906 convention he also prepared a report with F. O. Woodruff upon methods for the analysis of tea[6].

At this time, in his New York laboratory, Mr. Doolittle was working in active collaboration with two of his assistants—with A. F. Seeker upon methods for detecting the adulteration of maple products, on which two reports were presented at the 1908 meeting of the association[7], and with A. W. Ogden upon the composition of known samples of paprika[8]. The Doolittle-Ogden method for determining the iodine absorption of the non-volatile ether extract of paprika was reported upon at the 1908 meeting[9], and this work is still of importance owing to the long-continued practice of intensifying the color of inferior grades of paprika by grinding with olive oil. Among other subjects to which Mr. Doolittle gave attention during this period was that of the presence of tin in food products, and the Doolittle-Lourie method for determining tin in canned foods was discussed at the twenty-eighth annual convention[10].

Mr. Doolittle was elected a member of the Executive Committee of the association at the 1911 meeting, and from this time his attendance at the annual conventions was continuous. His previous activities as collaborator and referee were now to give way to work for the association in higher administrative capacities. In 1912 he was elected a member of the Standing Committee on Food Standards, and he continued in this office until 1914 when the committee was discharged with the establishment of the present Joint Committee on Food Standards and Definitions, composed of members of the Association of Official Agricultural Chemists, the Association of State Dairy, Food and Drug Officials, and the Bureau of Chemistry. Mr. Doolittle was appointed as one of the Bureau of Chemistry's representatives upon the Joint Committee in 1921, and he continued untiringly in the duties of this office up to the time of his death. The value of his extensive knowledge on the chemistry of foods to the work of this committee is incalculable, many of the standards and definitions of food that have been adopted being the direct result of his cooperation.

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[1] U S. Dept Agr Bur. Chem Bull. 81, p. 24
[2] Ibid., p. 30.
[3] Ibid., p. 33.
[4] U. S. Dept. Agr. Bur. Chem Bull 99, p 69.
[5] U. S. Dept Agr. Bur. Chem Bull 105, p 39.
[6] Ibid., p. 46
[7] U S Dept. Agr. Bur. Chem. Bull. 122, pp 196-199.
[8] *J. Am. Chem Soc.*, 1908, 30· 1481
[9] U S Dept Agr Bur. Chem. Bull 122, p 213.
[10] U S. Dept. Agr. Bur. Chem Bull. 152, p. 214.

At the 1914 meeting of the association Mr. Doolittle was elected chairman of the Committee on Editing Methods of Analysis, an appointment which he retained during the remainder of his life. To this work he devoted his best energies, never refusing to take upon himself the drudgery which others declined to accept. Much of this labor was performed during holidays and evenings in leisure moments which he might well have taken for rest and recreation. He frequently invited to his home or office small groups of chemists for conferences upon the improvement of analytical methods, and the writer has pleasant recollections of the discussion held with him upon the subject in company with those other two lamented members of the association—A. F. Seeker and A. H. Bryan. There are also other recollections of a several weeks' trip taken with Doolittle through the States of the Central District, when the manuscript of each chapter of *Methods of Analysis* was reviewed page by page on trains, in stations, and at hotels. It is a satisfaction that he lived to see the favorable reception that was universally accorded to the last edition of this book—a work which will remain a lasting monument of his industry and devotion to the work of the association.

In addition to the offices mentioned, Mr Doolittle had been a member of the Board of Editors of *The Journal* of the association since the time of its establishment in 1915 and also the Chairman of Subcommittee C since 1917. He was also Chairman of the Committee on Recommendations of Referees from 1920 to 1923, inclusive, and he served as president of the association at the fortieth annual convention in 1924. In all of these numerous activities he labored unceasingly for advancing the work of our organization.

Mr. Doolittle combined, in a manner which has never been surpassed, the double qualifications of chemical knowledge and regulatory efficiency. He was highly regarded in scientific circles, having been a member of the American Chemical Society, the American Association for the Advancement of Science, the American Pharmaceutical Society, and the American Public Health Association. In the work of all these organizations he took an active and important part.

The ethical ideals of Mr. Doolittle were of the very highest. Dr. Wiley, with whom he labored so faithfully in the early days of the enforcement of the Food and Drugs Act, has thus summarized his character: "There are few men that I have ever met who had a higher standard of ethics than Mr. Doolittle. He was especially devoted to what he believed to be the truth, fearless in his expressions and in his actions".

There was also in his make-up an element of the heroic, of which many of his closest friends were unaware. Although afflicted from early youth with an incurable malady, he never referred to this constant threat of his existence, but went courageously about his work smiling and uncomplaining—surrounding himself with an atmosphere of buoyant optimism that brought joy and encouragement to all with whom he came in contact. The late knowledge that perhaps he may have overspent his resources of strength in cheerfully assuming burdens which might have been done by others now comes as a reproach to all of us.

Accurate knowledge, rare administrative ability, fine judgment, and spotless integrity were traits of Mr. Doolittle that won everyone's respect, but the qualities which endeared him most to his many friends and co-workers were an indefinable gentleness and charm of manner that made him one of the best loved men in the wide circle of his acquaintances.

Among the papers found in Mr. Doolittle's desk, after his death, was a little poem, entitled "The Bridge Builder". The humane sentiment of its verses no doubt made a strong appeal to his generous nature. The poem exemplifies so perfectly the cardinal principle of his own philosophy of life, which was helpfulness to others, that it may well be chosen as a final tribute to his memory.

An old man, going a lone highway,
Came at the evening, cold and gray,
To a chasm vast and deep and wide,
The old man crossed in the twilight dim,
The sullen stream had no fear for him;
But he turned when safe on the other side
And built a bridge to span the tide.

"Old man", said a fellow pilgrim near,
"You are wasting your strength with building here;
Your journey will end with the ending day,
You never again will pass this way;
You've crossed the chasm deep and wide;
Why build you this bridge at evening tide?"

The builder lifted his old gray head—
"Good friend, in the path I have come", he said,
"There followeth after me today,
A youth whose feet must pass this way;
This chasm that has been as naught to me,
To that fair-haired youth may a pitfall be;
He, too, must cross in the twilight dim—
Good friend, I am building this bridge for him."

C. A. Browne.

PROCEEDINGS OF THE FORTY-SECOND ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1926.

The forty-second annual convention of the Association of Official Agricultural Chemists was held at the Willard Hotel, Washington, D. C., October 18-21, 1926.

The meeting was called to order by the president, W. W. Randall, Baltimore, Md., on the morning of October 18th, at 10 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER, 1927.

Honorary President.

Harvey W. Wiley, Mills Building, Washington, D. C.

President.

W. H. MacIntire, University of Tennessee, Knoxville, Tenn.

Vice-President.

Oswald Schreiner, Bureau of Plant Industry, Washington, D. C.

Secretary-Treasurer.

W. W. Skinner, Bureau of Chemistry, Washington, D. C.

Additional Members of the Executive Committee.

E. M. Bailey, New Haven, Conn.
L. D. Haigh, Columbia, Mo.

PERMANENT COMMITTEES.

Committee to Co-operate with Other Committees on Food Definitions.

Julius Hortvet (State Dairy and Food Commission, St. Paul, Minn.), *Chairman.*
C. D. Howard. E. M. Bailey.

Recommendations of Referees.

(Figures in parentheses refer to year in which appointment expires.)

SUB-COMMITTEE A: J. W. Kellogg (1928), (Department of Agriculture, Harrisburg, Pa.), *Chairman;* A. G. McCall (1930); R. N. Brackett (1932). [Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides (fluorine compounds); soils and liming materials (reaction value of soils, liming materials, less common metals in soils); feeding stuffs (stock feed adulteration, mineral mixed feeds, determination of moisture); sugars and sugar products (honey, maple products, starch conversion products, drying, densimetric and refractometric methods, polariscopic methods, chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, nitrogen activity methods in fertilizers, potash); plants (preparation of plant material for analyses, less common metals in plants, total chlorine in plants).]

SUB-COMMITTEE B: A. G. Murray (1928), (Bureau of Chemistry, Washington, D. C.), *Chairman;* L. E. Warren (1930); H. C. Lythgoe (1932). [Specific gravity and alcohol, spices and other condiments, naval stores (turpentine); drugs (alcohol in drugs, arsenicals, cocaine, chaulmoogra oil, crude drugs, chloroform and carbon tetrachloride, ipecac alkaloids, radio activity in drugs and water, laxatives and bitter tonics, mercurials, pyramidon, microchemical methods for alkaloids, silver proteinates, terpin hydrates, santonin, ether, bioassay of drugs).]

SUB-COMMITTEE C: C. F. Whitney (1928), (Laboratory of Hygiene, Burlington, Vt.), *Chairman;* H. A. Lepper (1930); E. M. Bailey (1932). [Dairy products (butter, cheese, malted milk, dried milk, ice cream, milk proteins, qualitative tests), fats and oils, baking powders and baking chemicals, eggs and egg products (total solids, detection of decomposition, water-soluble protein, unsaponifiable matter, and ash), food preservatives, coloring matters in foods, metals in foods (zinc in dried eggs), fruits and fruit products (ash in fruit products, fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat proteins), gelatin, cacao products (microscopical methods, crude fiber, cacao butter), cereal foods (sampling of flour, ash in flour and gasoline color value, glutenin in flour, hydrogen-ion concentration in flour, gluten in flour, diastatic value of flour, starch in flour, flour bleaching chemicals, methods for bread analysis, experimental baking tests, moisture in alimentary pastes, unsaponifiable matter and fat in flour and in alimentary pastes).]

Board of Editors.

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A. J. PATTEN (1928). R. B. DEEMER (1930).
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A. J. PATTEN. G. W. HOOVER.
W. H. MACINTIRE.

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G. S. Fraps. H. D. Haskins.

Committee on Revision of Methods of Soil Analysis.

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J. A. Bizzell. J. S. McHargue.

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H. J. Patterson, College Park, Md.

Committee on Sampling.

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J. W. Kellogg: *Foods and Feeding Stuffs.*
A. E. Paul: *Drugs.*
A. G. McCall: *Soils and Liming Materials.*
C. C. McDonnell: *Insecticides and Fungicides.*
R. N. Brackett: *Fertilizers.*
F. W. Zerban: *Saccharine Products.*
R. W. Frey: *Tanning Materials and Leathers.*
J. W. Sale: *Water.*
A. J. Patten: *Plants.*

Committee to Consider the Advisability of Studying Methods for the Analysis of Paint.

W. F. Hand (Agricultural and Mechanical College, Agricultural College, Miss.), *Chairman.*

W. T. Pearce. J. W. Kellogg.

Committee on Bibliography.

W. W. Skinner (Bureau of Chemistry, Washington, D. C.), *Chairman.*

G. S. Fraps. H. D. Haskins.
F. P. Veitch. W. W. Randall.

Committee on Constitution and By-Laws.

B. B. Ross (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman.*

E. M. Bailey. R. B. Deemer.
F. P. Veitch. W. W. Skinner.

Committee to Represent the Association at the First International Congress of Soil Science.

C. A. Browne, Washington, D. C.
W. W. Skinner, Washington, D. C.
W. H. MacIntire, Knoxville, Tenn.

REFEREES AND ASSOCIATE REFEREES.

WATERS, BRINE, AND SALT:
General referee: C. H. Badger, Bureau of Chemistry, Washington, D. C.

TANNING MATERIALS AND LEATHERS:
General referee: T. D. Jarrell, Bureau of Chemistry, Washington, D. C.

INSECTICIDES AND FUNGICIDES:
General referee: J. J. T. Graham, Bureau of Chemistry, Washington, D. C.

FLUORINE COMPOUNDS:
Associate referee: G. A. Shuey, Agricultural Experiment Station, Knoxville, Tenn.

SOILS AND LIMING MATERIALS:
General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

REACTION VALUE OF SOILS:
Associate referees: Alkaline soils, P. S. Burgess, Agricultural Experiment Station, Tucson, Ariz. Acid soils, E. T. Wherry, Bureau of Chemistry, Washington, D. C.

LIMING MATERIALS:
Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:
Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

FEEDING STUFFS:
General referee: W. F. Sterling, Bureau of Chemistry, Washington, D. C.

STOCK FEED ADULTERATION:
Associate referee: H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

MINERAL MIXED FEEDS:
Associate referee: H. A. Halvorson, 215 Old Capitol Building, St. Paul, Minn.

DETERMINATION OF MOISTURE:
Associate referee: G. E. Grattan, Department of Agriculture, Ottawa, Can.

SUGARS AND SUGAR PRODUCTS:
General referee: H. S. Paine, Bureau of Chemistry, Washington, D. C.

HONEY:
Associate referee: H. A. Schuette, Department of Chemistry, University of Wisconsin, Madison, Wis.

MAPLE PRODUCTS:
Associate referee: H. M. Lancaster, 317 Queen St., Ottawa, Can.

STARCH CONVERSION PRODUCTS:
Associate referee:

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:
Associate referee: R. T. Balch, Bureau of Chemistry, Washington, D. C.

Polariscopic methods:
Associate referee: F. W. Zerban, N. Y. Sugar Trade Laboratory, 80 South St., New York City.

Chemical methods for reducing sugars:
Associate referee: R. F. Jackson, Bureau of Standards, Washington, D. C.

Fertilizers:
General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Texas.

Phosphoric acid:
Associate referee: W. H. Ross, Bureau of Soils, Washington, D. C.

Nitrogen:
Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

Nitrogen activity methods in fertilizers:
Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

Potash:
Associate referee: A. P. Kerr, Agricultural Experiment Station, Baton Rouge, La.

Plants:
General referee: A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

Preparation of plant material for analysis:
Associate referee: H. R. Kraybill, Agricultural Experiment Station, Purdue, Ind.

Less common metals in plants:
Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

Total chlorine in plants:
Associate referee: Doris Tilden, U. S. Food and Drug Inspection Station, San Francisco, Calif.

Specific gravity and alcohol:
General referee: A. W. Hanson, U. S. Food and Drug Inspection Station, Minneapolis, Minn.

Spices and other condiments:
General referee: W. C. Geagley, Food and Drug Department, Lansing, Mich.

Naval stores:
General referee: F. P. Veitch, Bureau of Chemistry, Washington, D. C.

Turpentine:
Associate referee: V. E. Grotlisch, Bureau of Chemistry, Washington, D. C.

Drugs:
General referee: A. E. Paul, U. S. Food and Drug Inspection Station, Chicago, Ill.

Alcohol in drugs:
Associate referee: C. D. Howard, Agricultural Experiment Station, Concord, N. H.

Arsenicals:
Associate referee: H. Wales, Bureau of Chemistry, Washington, D. C.

Cocaine:
Associate referee: E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif.

Chaulmoogra oil:
Associate referee: L. E. Warren, Bureau of Chemistry, Washington, D. C.

Crude drugs:
Associate referee: J. F. Clevenger, Bureau of Chemistry, Washington, D. C.

Chloroform and carbon tetrachloride:
Associate referee: H. O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.

Ipecac alkaloids:
Associate referee: A. R. Bliss, University of Tennessee, Memphis, Tenn.

Radio activity in drugs and water:
Associate referee: J. W. Sale, Bureau of Chemistry, Washington, D. C.

Laxatives and bitter tonics:
Associate referee: M. M. Woodward, Department of Agriculture, Lansing, Mich.

Mercurials:
Associate referee: P. W. Morgan, U. S. Food and Drug Inspection Station, Chicago, Ill.

Pyramidon:
Associate referee: F. L. Elliott, U. S. Food and Drug Inspection Station, Boston, Mass.

Microchemical methods for alkaloids:
Associate referee: C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.

Silver proteinates:
Associate referee: L. Jones, U. S. Food and Drug Inspection Station, Chicago, Ill.

Terpin hydrate:
Associate referee: C. W. Harrison, U. S. Food and Drug Inspection Station, Baltimore, Md.

Santonin:
Associate referee: E. L. Redfern, Agricultural Experiment Station, Des Moines, Iowa.

Ether:
Associate referee: G. C. Spencer, Bureau of Chemistry, Washington, D. C.

Bioassay of drugs:
Associate referee: J. C. Munch, Bureau of Chemistry, Washington, D. C.

Dairy products:
General referee: Julius Hortvet, Dairy and Food Department, St. Paul, Minn.

Butter:
Associate referee: L. C. Mitchell, U. S. Food and Drug Inspection Station, St. Louis, Mo.

CHEESE:
Associate referee: E. O. Huebner, Dairy and Food Commission, Madison, Wis.

DRIED MILK:
Associate referee: J. T. Keister, Bureau of Chemistry, Washington, D. C.

MALTED MILK:
Associate referee: B. G. Hartmann, Bureau of Chemistry, Washington, D. C.

ICE CREAM:
Associate referee: L. H. McRoberts, Food and Drug Laboratory, Bismarck, N. Dak.

MILK PROTEINS:
Associate referee: H. C. Waterman, Bureau of Chemistry, Washington, D. C.

QUALITATIVE TESTS:
Associate referee: S. H. Hall, Department of Public Health, Boston, Mass.

FATS AND OILS:
General referee: G. S. Jamieson, Bureau of Chemistry, Washington, D. C.

BAKING POWDERS AND BAKING CHEMICALS:
General referee: L. H. Bailey, Bureau of Chemistry, Washington, D. C.

EGGS AND EGG PRODUCTS:
General referee: J. C. Palmer, Bureau of Chemistry, Washington, D. C.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH:
Associate referee: Samuel Alfend, U. S. Food and Drug Inspection Station, St. Louis, Mo.

DETECTION OF DECOMPOSITION:
Associate referee: H. I. Macomber, U. S. Food and Drug Inspection Station, New York, N. Y.

TOTAL SOLIDS:
Associate referee: J. C. Palmer, Bureau of Chemistry, Washington, D. C.

FOOD PRESERVATIVES:
General referee: W. W. Randall, State Department of Health, Baltimore, Md.

COLORING MATTERS IN FOODS:
General referee: C. F. Jablonski, U. S. Food and Drug Inspection Station, New York City.

METALS IN FOODS:
General referee: W. F. Clarke, Bureau of Chemistry, Washington, D. C.

ZINC IN DRIED EGGS:
Associate referee: W. E. Kirby, U. S. Food and Drug Inspection Station, New York City.

FRUITS AND FRUIT PRODUCTS:
General referee: H. J. Wichmann, U. S. Food and Drug Inspection Station, San Francisco, Calif.

FRUIT ACIDS:
Associate referee: E. K. Nelson, Bureau of Chemistry, Washington, D. C.

ASH IN FRUIT PRODUCTS:
Associate referee: Doris Tilden, U. S. Food and Drug Inspection Station, San Francisco, Calif.

CANNED FOODS:
General referee: I. L. Miller, State Food and Drug Department, Indianapolis, Ind.

CEREAL FOODS:
General referee: F. C. Blanck, Bureau of Chemistry, Washington, D. C.

SAMPLING OF FLOUR:
Associate referee: H. Runkel, U. S. Food and Drug Inspection Station, Minneapolis, Minn.

ASH IN FLOUR AND GASOLINE COLOR VALUE:
Associate referee: D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

GLUTENIN IN FLOUR:
Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

H-ION CONCENTRATION OF FLOUR:
Associate referee: C. H. Bailey, University of Minnesota, Minneapolis, Minn.

GLUTEN IN FLOUR:
Associate referee: Raymond Hertwig, Hecker-H-O Co., Genesee Building, Buffalo, N. Y.

DIASTATIC VALUE OF FLOUR:
Associate referee: E. L. Tague, State Agricultural College, Manhattan, Kans.

STARCH IN FLOUR:
Associate referee: J. C. Palmer, Bureau of Chemistry, Washington, D. C.

FLOUR BLEACHING CHEMICALS:
Associate referee: G. C. Spencer, Bureau of Chemistry, Washington, D. C.

METHODS FOR BREAD ANALYSIS:
Associate referee: J. C. Palmer, Bureau of Chemistry, Washington, D. C.

EXPERIMENTAL BAKING TESTS:
Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

MOISTURE IN ALIMENTARY PASTES:
Associate referee: G. C. Spencer, Bureau of Chemistry, Washington, D. C.

UNSAPONIFIABLE MATTER AND FAT IN FLOUR AND IN ALIMENTARY PASTES:
Associate referee: Samuel Alfend, U. S. Food and Drug Inspection Station, St. Louis, Mo.

VINEGARS:
General referee: J. O. Clarke, U. S. Food and Drug Inspection Station, New York City.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
General referee: J. W. Sale, Bureau of Chemistry, Washington, D. C.

MEAT AND MEAT PRODUCTS:
General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:
Associate referee: W. S. Ritchie, Department of Agricultural Chemistry, University of Missouri, Columbia, Mo.

GELATIN:
General referee: E. H. Berry, U. S. Food and Drug Inspection Station, Chicago, Ill.

CACAO PRODUCTS:
General referee: H. A. Lepper, Bureau of Chemistry, Washington, D. C.

MICROSCOPIC METHODS:
Associate referee: V. A. Pease, Bureau of Chemistry, Washington, D. C.

CRUDE FIBER:
Associate referee: E. R. Miller, U. S. Food and Drug Inspection Station, New York, N. Y.

CACAO BUTTER:
Associate referee: L. W. Ferris, U. S. Food and Drug Inspection Station, Buffalo, N. Y.

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Smith, J. B., Experiment Station, Kingston, R. I.
Smith, Miss K. A., Bureau of Chemistry, Washington, D. C.
Smith, P. H., Feed Stuff Control, Amherst, Mass.
Smith, S. L., Office of Experiment Stations, Washington, D. C.
Snyder, E. F., Bureau of Plant Industry, Washington, D. C.
Soule, A. M. G., Department of Agriculture, Augusta, Me.
Spencer, Mrs. G. C., 501 Dorset Ave., Chevy Chase, Md.
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Stanback, J. F., Department of Agriculture, Raleigh, N. C.
Stengel, A., Bureau of Chemistry, Washington, D. C.
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Stokes, W. E., Royal Baking Powder Co., Brooklyn, N. Y.
Strauss, A. C., Kuttroff, Pickhardt & Co., Inc., New York, N. Y.
Strowd, W. H., Soft Wheat Millers Association, Nashville, Tenn.

Taylor, A. E., Bureau of Chemistry, Washington, D. C.
Temple, W. G., Temple & Co., Norfolk, Va.

Thom, C., Bureau of Chemistry, Washington, D. C.
Thompson, E. C., 350 Madison Ave., The Borden Co., New York, N. Y.
Thornton, E. W., R. B. Davis Co., Hoboken, N. J.
Tobey, E. R., Agricultural Experiment Station, Orono, Me.
Toll, J. D., The American Fertilizer, Philadelphia, Pa.
Treuthardt, E. L. P., Bureau of Chemistry, Boston, Mass.
Turner, J. D., Department of Feed Control, Lexington, Ky.
Turrentine, J. W., Bureau of Soils, Washington, D. C.

Veitch, F. P., Bureau of Chemistry, Washington, D. C.
Vollertsen, J. J., Armour & Co., Chicago, Ill.

Wales, H., Bureau of Chemistry, Washington, D. C.
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Ward, K. Jr., Bureau of Chemistry, Washington, D. C.
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Watkins, H. R., Bureau of Chemistry, Washington, D. C.
Webber, Mrs. W. P., Rochester, N. Y.
Webber, W. P., Rochester, N. Y.
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Wilson, S. H., Department of Agriculture, Atlanta, Ga.
Wilson, S. M., The Baugh Chemical Co., Baltimore, Md.
Woods, A. F., Department of Agriculture, Washington, D. C.

Zerban, F. W., N. Y. Sugar Trade Laboratory, N. Y. City.

PRESIDENT'S ADDRESS[1].

THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS AND THE PURE FOOD MOVEMENT.

By WYATT W. RANDALL (State of Maryland Department of Health, Baltimore, Md.).

We are met together to-day as a group of organizations whose common interests are many, whose several achievements have been notable, and whose high ideal has been upheld. Among these common interests are the provision, at no excessive cost, of wholesome food and drink for our vast population and the development of the agricultural resources of our country for the benefit of all our people. Among our several achievements have been the fostering of the sense of responsibility, on the part of officials and of legislatures—national and local alike—for the health of the population, and the formulation of means whereby the public may, in certain important fields, be protected from exploitation. Our single ideal has been to promote in our special field, and to make ever more nearly universal, honorable dealing between man and man.

The special purpose of our joint meeting is to celebrate the twentieth anniversary of the passage of the Food and Drugs Act of 1906. We are therefore aiming to bring to the mind of the public how many and great have been the benefits which have flowed from that outstanding piece of legislation. While others, more competent than I, will speak on the new era which it inaugurated, I propose, with your permission, to comment upon certain phases of the early history of the association I have the honor to represent, and upon the influence which that organization may be said to have exerted in preparation for the food law administration of to-day.

In order that I may not put implications into the words of others to which they would not subscribe, I shall make use, in considerable measure, of the actual words which they employed.

Along with the signal contributions of Dr. Harvey Wiley to the advancement of science as applied to agriculture and to our information concerning agricultural products, we owe him a debt of gratitude for the series of papers he has written and the reminiscences he has poured forth dealing with the early history of the Association of Official Agricultural Chemists. Unlike the ingenuous historian of the New England colonies who began his chronicle with an account of the Flood and whose long life and indefatigable industry did not suffice to bring the record down to the sailing of the "Mayflower", our honorary president wisely decided to describe conditions as they existed just before this association came into being and, having thus established a point-from-which, he took up the tale of events as he had himself observed them and had participated in their shaping. In a paper read before the association meeting in 1899, Dr. Wiley says:

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[1] Presented Wednesday morning, October 19th, as a part of the general program of the joint meeting of the Association of Official Agricultural Chemists, the Association of Dairy, Food and Drug Officials and the Association of Feed Control Officials

"The condition of agricultural chemical work in the United States in 1880 was a peculiar one. The few chemists who were engaged in agricultural research were acting in complete independence of each other in regard to methods of investigation and of research. Some of them were using the methods employed by German chemists, while others followed the instruction given by the French or English agricultural chemists. There was no unity of interest in the profession nor any common system of work. The condition of analytical work may truly be described as chaotic. The result of such condition [*sic*] is easily imagined. There was no standard of comparison or reference. Buyers and sellers were continually wrangling over analyses, which, made by different men following different methods, did not agree.

"The sellers' chemists uniformly obtained higher results than the buyers', and thus the door to litigation was constantly open.

"Strange as it may seem, the first steps toward correcting this pitiable condition did not come from the Department of Agriculture at Washington, but from the department of agriculture of one of the States. It was through the Hon. J. T. Henderson, commissioner of agriculture of Georgia, and at the instigation of Mr. H. J. Redding, now [1899] director of the Georgia station, that the first step toward uniformity of action among agricultural chemists of the United States was taken."

In response to an invitation issued by Judge Henderson, 20 persons interested in the matter of fertilizer analysis and control assembled in Washington in July, 1880. After some discussion, a committee of five was appointed to draft tentative methods for the estimation of phosphoric acid in its several forms, and a similar committee, to draft tentative methods for the estimation of nitrogen (including nitrates) and potash. It was proposed that the members of this convention form themselves into a subsection of the division of chemistry in the American Association for the Advancement of Science, and a committee was appointed to make the necessary arrangements. The question of a method for arriving at commercial valuations was discussed but was postponed, to be considered at the adjourned meeting to be held a few weeks later in Boston. Here, as at Washington, the plan for incorporating as a subsection of the American Association for the Advancement of Science met with favor. Those in attendance ("about 25") pledged themselves to try out the several analytical methods proposed at the Washington meeting.

In August, 1881, the fertilizer chemists met with the A. A. A. S. at Cincinnati. The minutes list by name 32 chemists as among those who were in attendance. There was evidently much controversy over rival methods for the determination of insoluble phosphoric acid; as is often done in such cases, the convention referred the matter to a committee and then adjourned. Dr. Wiley comments as follows: "After the adjournment of the Cincinnati meeting the interest in the collaboration of the agricultural chemists seemed to die out. There was a certain feeling of antipathy—perhaps it is not well to make it so strong as this, but a strong feeling of incongruity—existing between the trade chemists on the one hand and the official chemists on the other. It was an unvoiced sentiment pervading the organization to the effect that an association composed of trade chemists and official chemists contained elements of instability which would prevent it from ever becoming highly useful. Nevertheless, after a lapse of three years, Mr. Henderson again called a meeting, which was held at Atlanta, Ga."

This meeting (May, 1884) was attended by 30 chemists and others interested in the matters at issue. Various reports were read and criticized; amendments were offered and, ultimately, approved methods for the analysis of fertilizers were formulated, subsequently to be published in a bulletin. The meeting then adjourned to convene once more at Philadelphia in the following September in conjunction with the A. A. A. S.

Once more I quote from Dr. Wiley's account:

"The Philadelphia meeting was held September 8, 1884. Dr. E. H. Jenkins was appointed chairman and Dr. C. W. Dabney acted as secretary. A committee appointed at the Atlanta meeting to consider the advisability of organizing the association as a subsection of the American Association for the Advancement of Science recommended the formation of two associations.

"*First.* The Association of Agricultural Chemists to be entirely distinct from the American Association, [and] to which should be left the discussion of the methods of analysis, etc.

"*Second.* The subsection of the American Association for the Advancement of Science to be open to all agricultural chemists for the purpose of investigation and discussion.

"The unanimous opinion expressed in the discussion of this subject was that an organization entirely separate from the American Association would best advance the objects of the convention. A committee, consisting of Messrs. H. C. White, E. H. Jenkins, P. C. Chazal, J. A. Myers, and H. W. Wiley, was appointed to consider the form of organization and instructed to report the following day. The report of this committee, with a few later additions, forms the constitution of the association as it exists to-day [1899].

"On September 9 a formal organization took place, the present name of the association was adopted, officers for the following year were elected, and the convention resolved into the first annual meeting of the Association of Official Agricultural Chemists. Committees on phosphoric acid, potash, and nitrogen were appointed, and methods for the determination of phosphoric acid and potash in commercial fertilizers adopted as the official methods of the association.

"The bulletin containing the proceedings of this meeting consists of eight pages of printed matter, in which are given the list of officers elected, the constitution, and the methods of analysis adopted officially by the association."

So much for the origin of the association and of its name. Now let us consider the development of some of its characteristics as we note them to-day.

The Fall meeting of 1884 is thus reckoned the first annual meeting of the association; we are now in the midst of the forty-second, for in 1918 no meeting was held. Gradually, as the years pass, a larger and larger attendance is to be noted, more topics are added to the list, and a greater volume of printed material is issued in the form of proceedings and approved methods. The handful of chemists that met in 1884 has been succeeded by the 287 in attendance in 1925; the eight-page bulletin of 1884 contrasts with the (possibly) 500 pages of proceedings and contributed papers of 1925, and the revised book of standard methods.

I wish now to call your attention to one of the first signs I have noted, in glancing over the record of proceedings of those earlier years, of a new interest developing. Thus, President Huston in his address at the Twelfth Convention (1895) remarks:

"A young lady recently returned from the blue-grass region, on being asked for her impression of the State, said that it seemed to be a place where they educated their horses and let the men just grow up. This association seems to be in a somewhat analogous position on the food question. For, while our reports abound in researches on food for live stock, the only work on the food for man is represented in the reports on dairy products and sugar, and as a rule comparatively few of us contribute to either of these branches. Even the question of hotel accommodations was referred to the Committee on Fermented and Distilled Liquors * * *. The association might extend its usefulness by giving some attention to the subject of the examination of many kinds of human food, which we all know will hardly bear close scrutiny."

It will be a source of some astonishment to many of the younger members of the association that only after eleven years of active life was the careful examination of articles of human food suggested as possibly another, and a proper, field of work for the members of the A. O. A. C.

When one reads the proceedings of the meeting of 1896, he is likely to see something bordering on the comic in a recommendation adopted near the close of that session. It reads: "(4) That methods for detecting the adulteration of foods, so far as such are not provided for in connection with other reporters, be referred to the reporter on spirituous liquors". On its adoption Mr. Winton—shall I say, dryly—remarked: "It appears that this reporter on liquors has had an easy time of it, although he has distributed bottles, but no one seems to have had any time for analytical work * * *. I would therefore move that the duty of the reporter on fermented and distilled liquors be increased, and that he may be made the reporter of methods for the detection of adulterants in food". And Mr. Frear followed with: "I would like to say regarding food adulterations that I have had a little work to do in that line. I wish we had had a reporter on the subject five years ago. I would be heartily glad if the association in some form would provide for beginning work in this direction. It seems to me a proper subject for the association to undertake, since it is a matter of more than ordinary importance".

The suggestion made by Huston soon began to bear fruit. In 1897 the association appointed five members who were to constitute "a committee on food standards to consider the practicability of compiling, for the use of the increasing proportion of the association's membership charged with the duty of official food control, all accessible and trustworthy chemical and physical data as to the composition of foods and drinks for man and domestic animals offered for sale on the American market".

Next year (1898) President Winton took up this question anew, and with an increased confidence which indicates the development of the idea. He says:

"During the first two years of its existence our organization was really a body of fertilizer analysts, as only methods for the analysis of these materials were considered; but at the third convention the constitution was amended so as to include as subjects for investigation 'The analysis of soils, cattle foods, dairy products, and other materials connected with agricultural industry'. The subjects fodders and dairy products were referred that year (1886) to committees, and sugar and distilled liquors in the following year. The first reporter on soils and ash was appointed in 1890 and on tannin in 1894. The methods for the analysis of dairy products and liquors which were tested included those for the detection of adulteration, and in 1896 this line of investigation was extended so as to cover all articles used for food and drink, a task which bids fair to become one of the most important we have yet undertaken".

Yet even Winton seems to have been more cautious than most prophets, for, after advocating the study of fungicides and insecticides as a coming field for the analyst, he remarks: "There is no immediate prospect that a further expansion of our work will be found advisable".

Further on in his address, Winton says: "The action taken at the last convention [i. e. that of 1897], leading to the appointment of a committee to consider standards of purity of the foods and drinks on sale in the United States, was but another manifestation of the growing determination to suppress adulteration and misbranding. The adoption of standards of purity should go hand in hand with the adoption of methods of examination, as the proper interpretation of results is as essential as the accuracy of their determination".

You will realize, I am sure, that the quotations made from Huston's and Winton's addresses indicate that the movement was under way which was to result in the Federal Food and Drugs Act of 1906, in the Meat Inspection Act of 1906-7, and in various other laws and regulations, Federal, State and municipal, affecting the production, distribution, and sale of foods.

Going forward two more years, we find that President Kilgore, in 1900, comments upon the situation as follows:

"Not only has the scope of our work been extended, but the influence of the association has grown. During the year we have been called upon as an organization to aid in the education of public sentiment and to bring influence to bear in favor of a national pure food bill, with which we are all more or less familiar. Knowing that a number of the members of the association are actively engaged in the examination of food products and aiding to execute State pure-food laws, and feeling that our entire membership is deeply interested in this important movement upon which so much of health and happiness for ourselves and those we represent depends, I used the name of the association and joined other scientific bodies in this country in asking our Congress to pass a national pure-food law. I trust that I may have the approval of the association in this action."

Further on he says:

"* * * May I in a few words draw your attention to the growing prominence and importance of the examination of human food and drink? Recently a number of States have passed pure-food laws. We are in need of new methods and of concerted tests of old ones for the examination of food products * * *. Would it not be well for the association to make a number of subdivisions of this subject, and place experienced men in charge of the investigation of each subdivision?"

At this meeting (1900) it was decided to arrange for the appointment of fifteen associate referees who should, each in a special field, assist the

Referee on Liquor and Food Adulteration in the work of investigation. The committee on pure-food standards, in addition, presented a resolution which was adopted and which reads (in part) as follows:

"*Resolved*, That the Association of Official Agricultural Chemists of the United States hereby reaffirms its conviction of the urgent necessity for national legislation on broad lines for the prevention of adulteration of foods and drugs that are subjects of interstate commerce and commerce with foreign lands, for the better protection of the health of our citizens and the legitimate interests of producers and dealers, and for the national reputation of business honor, and most earnestly and respectfully urges upon the Congress of the United States the present enactment of the measure now on the Calendar of the House of Representatives with a favorable recommendation, after long and careful consideration of the Committee on Interstate and Foreign Commerce * * *."

My object in noting the increase, during the first twenty years of the life of this association, in the interest displayed in the problem of protecting the community at large from adulterated and misbranded foods must, by this time, be obvious. The passage in 1906 of the national Food and Drugs Act was in no small measure the result of the efforts of this association as a body and, yet more particularly, of the zeal of our honorary president. It was gradually brought home to members of the Congress and to State authorities that there existed a crying need, to meet which in satisfactory fashion the nation and its integral parts were in honor—nay, in common decency—unquestionably bound. The knowledge upon which was based the demand for corrective legislation, and without which no pressure could have been employed for its enactment, was in large measure furnished by the patient work of members of this association through those twenty useful years.

But this is not all. The need for legislation being granted, there remained the question of its effective enforcement. Would it be possible so to present the case of the public in court that the object of the Food and Drugs Act could be attained? Did standards exist, or could standards be set up, by which food products could be definitely classified as genuine or, on the other hand, as sophisticated? Did methods of analysis exist, or could such methods be devised, by which, to the satisfaction of all parties concerned, the true nature of the products in question could be established? That these questions can, in the great majority of cases, be answered in the affirmative is largely a result of the labors of this association. If the tools were not already at hand, the way to produce them was indicated; if the workers were too few or were inexperienced, the kind of skill called for and the methods by which it could be secured were in evidence.

It would seem appropriate, therefore, that at this meeting, when the twentieth anniversary of the passage of the Food and Drugs Act is commemorated, due praise be given to those who, in its early days, trained the Association of Official Agricultural Chemists for the work it was ultimately to be called upon to perform. That they grasped otherwise than partially what the future held in store, I do not contend. Their outstanding merit lies in their determination that the efforts of the association—whatever the object immediately in view—should always be so directed that accurate data, and these only, should form their harvest and should be garnered for the benefit of mankind.

Before I close, there is one matter concerning which I would offer a suggestion. This being a joint meeting, the points of contact with our fellow associations and the boundaries that separate us naturally come into our minds. The Association of Official Agricultural Chemists is essentially a group of experts, a society whose specific *raison d'être* is the critical study of methods of analysis as fast as they may be suggested, their improvement as experience may dictate, and their establishment as means of arbitration and for providing information for courts of justice. It is difficult to imagine any other agency that—at least, in theory—could better achieve these useful ends. That there is need for such an organization is not open to question; our problem is to improve the one now in existence or, on the other hand, to substitute another which promises to be more efficient. I think it is but fair to say that, with such experience as we have had, no other type of agency seems likely to be more successful. In so far as there has been failure to hit the mark aimed at, such failure has been due, not to the form of organization, but to the very human characteristics of its individual members and to the conditions under which they live and move and make their living. An association of hard-worked men, which must depend for its results upon the voluntary labor given by its members—often at considerable cost and with no monetary return—must, in the nature of the case, face no little disappointment. That the achievement has been so marked and the disappointments relatively so few, may well stir within us a worthy pride.

Can two or more agencies accomplish this work better than one? Hardly. A single clearing-house for handling such investigation, a single publishing medium, a single official collection of approved specifications, must in the nature of the case make for economy of effort and of expense, and for finality. The benefits possible from competition and from wide variety of working conditions can be secured as well within an association like ours as from two or more organizations with practically similar aims. Concentration by each group primarily upon a special work: Public health men upon the problems peculiar to general sanitation, water and sewage engineers upon those of water supply and waste disposal, pharmacologists and pharmacists upon those peculiar to drug preparation and administration, feed officials upon those peculiar to the administration of feed laws, food officials upon those connected with the administration of the Food and Drugs Act, and, finally, the A. O. A. C. upon those involving analysis; such a division must seem reasonable. Not that hard and fast boundaries can always be drawn, but at least duplication can be avoided. In each field there should be but a single court of last resort. By all means let investigation be furthered wherever the need for it finds expression and the capacity and the willingness to do it exist, but equally, by all means, let the results be passed on to that organization in whose special field the matter belongs and where they will meet with sympathetic criticism.

I would ask, therefore, of members of the associations other than that for which I may assume to speak, that they communicate to us reports of their difficulties and of their successes—in so far as these involve analytical matters—and we, in our turn, will gladly turn over for consideration by those more competent than ourselves to pass upon them, questions of administration and such scientific data as, coming under notice, seem rather to be a part of the heritage of organizations other than ours.

ORDER OF PUBLICATION.

The reports of the committees presented on the last day of the annual meeting will be given at the beginning of the proceedings rather than in their chronological order. This will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

FOURTH DAY.

THURSDAY -MORNING SESSION.

REPORT OF THE REPRESENTATIVE AT THE NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH.

The fifth annual meeting of the National Conference on Pharmaceutical Research was held at the Hotel Bellevue-Stratford, Philadelphia, Pa., on Saturday, September 11, 1926, just prior to the annual meeting of the American Pharmaceutical Association.

Sessions held in the morning and afternoon were attended by about sixty representatives of the leading pharmaceutical organizations of this country. Delegates were present from the following fourteen organizations:

American Association of Colleges of Pharmacy;
American Chemical Society, Division of Chemistry of Medicinal Products;
American Drug Manufacturers Association;
Association of Official Agricultural Chemists;
American Pharmaceutical Association;
American Pharmaceutical Manufacturers Association;
Bureau of Chemistry, U. S. Department of Agriculture;
National Association of Boards of Pharmacy;
National Association of Retail Druggists;
National Formulary Revision Committee;
Pharmaceutical Laboratory Seminar;
Plant Science Seminar;
The Proprietary Association; and
U. S. Pharmacopeia Revision Committee.

In addition, an unofficial observer was present from the Mellon Institute.

Report was made by the Chairman, H. V. Arny, that the census of pharmaceutical research of 1926 was completed and published in the *J. Am. Ph. Assoc.*, Vol. XV, No. 8, p. 690-700.

Brief reports of progress were made by some of the chairmen of the ten standing committees, detailed reports being made by the following representatives:

L. F. Kebler, H. A. B. Dunning, H. W. Youngken, E. L. Newcomb, H. V. Arny, G. D. Beale, and Arno Viehoever.

Announcement was made that the American Therapeutic Association had declined to become affiliated with the Conference; also that it was deemed advisable for consideration to be given to including the following as affiliated members:

Hygienic Laboratory, Public Health Service, U. S. Treasury Department.
Mellon Institute.
Sprague Institute.

Consideration was given to a number of recommendations included in the chairman's address. The conference decided that an increase of funds for pharmaceutical research was desirable, but that no efforts were necessary now to obtain assistance in certain directions, nor for the encouragement of potential workers. Decision was made to continue to publish results reached by the Conference in the Journal of the American Pharmaceutical Association without any expense to the conference, and that as a result of this decision no more funds were needed for running expenses.

The committee appointed to consider the advisability of publishing a book describing the research achievements of pharmacy recommended that the American Pharmaceutical Association arrange to publish such a book in a popular style, a suggested list of subjects and their authors being included. The conference recommended that the subject be referred to the Council of the American Pharmaceutical Association for consideration.

The Nominating Committee presented the following names for officers for 1926-27: Chairman, H. V. Arny of New York; Vice Chairman, J. H. Webster of Detroit; Secretary-Treasurer, J. C. Krantz, Jr. of Baltimore, all of whom were duly elected.

The question of holding meetings of the Conference at other times and places, instead of in conjunction with the annual meeting of the American Pharmaceutical Association, was considered, and decision was reached that in the future the Research Conference should meet in the same city as, and on the Saturday preceding, the annual meeting of the American Pharmaceutical Association.

C. S. BRINTON.

Approved.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FORTY-SECOND ANNUAL CONVENTION, OCTOBER 18-21, 1926[1].

I. FERTILIZERS.

(1) The calcium chloride method for the preparation of ammonium citrate solution (p. 4, **13** (2)) was dropped (first action).

(2) The words "dilute to 1 liter" in the second of the alternative methods for the preparation of magnesia mixture (p. 2, **5** (**c**)), last line, were changed to read "proceed as in (1)".

(3) The absolute or cupric oxide method for the determination of total nitrogen (p. 9) was dropped (first action).

II. SOILS.

No additions, deletions, or other changes.

III. AGRICULTURAL LIMING MATERIALS.

No additions, deletions, or other changes.

IV. PLANTS.

No additions, deletions, or other changes.

V. INSECTICIDES AND FUNGICIDES.

(1) The official method for the determination of cyanogen in sodium and potassium cyanides (p. 65) was dropped (first action).

(2) The official method for the determination of chlorine in sodium and potassium cyanides (p. 65) was dropped (first action).

(3) The following method for the determination of cyanogen in sodium and potassium cyanides was adopted as official (first action):

CYANOGEN.—TENTATIVE.

REAGENTS.

(**a**) *0.1 N silver nitrate solution.*—Standardize against pure sodium chloride by titration, using chromate indicator; or gravimetrically, weighing the chloride.

(**b**) *Lead carbonate.*

(**c**) *Sodium hydroxide solution.*—Dissolve 100 grams of sodium hydroxide in water and dilute to 1 liter.

(**d**) *Potassium iodide.*—Crystals, or a saturated solution.

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[1] As compiled by the Committee on Editing Methods of Analysis, R W Balcom, Chairman. Unless otherwise stated, all references in this report are to *Methods of Analysis, A O A C*, 1925.

DETERMINATION.

Break the sample into small lumps in a mortar (do not grind). Weigh quickly about 5 grams in a weighing bottle and wash into a 500 cc. volumetric flask containing about 200 cc. of water. Add a little lead carbonate to precipitate any sulfide sulfur that may be present, dilute to the mark with water, mix thoroughly, filter through a dry filter, and then transfer a 50 cc. aliquot to a 400 cc. beaker. Add 200 cc. of water, 5 cc. of the sodium hydroxide solution, and 10 drops of the potassium iodide solution (or a few crystals) and titrate to a faint opalescence with the 0.1 *N* silver nitrate solution. (In making this titration, it is advantageous to have the beaker over a black surface.) From the number of cubic centimeters of 0.1 *N* silver nitrate used calculate the percentage of cyanogen (CN) in the sample. The reaction is represented by the equation

$$2\ NaCN + AgNO_3 = NaCN\ AgCN + NaNO_3;$$

hence, 1 cc. of 0.1 *N* silver nitrate solution is equivalent to 0.005202 gram of cyanogen.

(4) The following methods for the determination of chlorine in sodium and potassium cyanides were adopted as official (first action):

CHLORINE.—TENTATIVE.

REAGENTS.

(a) *0.1 N silver nitrate solution.*—Prepare as directed under cyanogen in sodium and potassium cyanides, Reagent (a).

(b) *0.1 N ammonium or potassium thiocyanate solution.*—Adjust by titrating against the 0.1 *N* silver nitrate solution.

(c) *Formaldehyde solution.*—A 40 per cent chloride-free solution.

(d) *Ferric indicator.*—A saturated solution of ferric ammonium alum.

DETERMINATION.

Method I.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a beaker, dilute with an equal volume of water, add 1 to 2 cc. of the formaldehyde solution, stir well, and let stand for 15 minutes. Acidify with nitric acid (5 cc. of 1+1 is usually enough), add a measured volume of the 0.1 *N* silver nitrate solution sufficient to give an excess, stir well, filter, wash, and titrate the excess of silver in the combined filtrate and washings with the 0.1 *N* thiocyanate solution, using ferric indicator. From the number of cubic centimeters of 0.1 *N* silver nitrate solution, less the number of cubic centimeters of 0.1 *N* thiocyanate solution used, calculate the percentage of chlorine in the sample.

Method II.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a distilling flask, dilute to 100 to 150 cc., acidify with a slight excess of acetic acid, and distil, passing the vapors through a condenser, the delivery end of which dips into a solution of sodium hydroxide, to absorb the hydrocyanic acid. After all the hydrocyanic acid has been driven off, which should be the case when 50 cc. of distillate has passed over, wash the liquid remaining in the distilling flask into a beaker, add 5 cc. of dilute nitric acid (1+1) and then a measured volume of the 0.1 *N* silver nitrate solution sufficient to give an excess, stir well, filter, wash, and titrate the excess of silver in the combined filtrate and washings with the 0.1 *N* thiocyanate solution, using ferric indicator. From the number of cubic centimeters of 0.1 *N* silver nitrate solution, less the number of cubic centimeters of 0.1 *N* thiocyanate solution used, calculate the percentage of chlorine in the sample.

(5) The following method for the determination of cyanogen in calcium cyanide was adopted as an official method (first action):

CYANOGEN.—TENTATIVE.

REAGENTS.

(**a**) *0.1 N silver nitrate solution.*—Prepare as directed under cyanogen in sodium and potassium cyanides, Reagent (**a**).

(**b**) *Soda-lead reagent*—Dissolve 20 grams of lead acetate in water, dilute to 1 liter, and add 200 grams of chloride-free sodium carbonate.

(**c**) *Sodium hydroxide solution.*—Prepare as directed under cyanogen in sodium and potassium cyanides, Reagent (**c**).

(**d**) *Potassium iodide.*—Crystals, or a saturated solution.

DETERMINATION.

Place about 200 cc. of water in a 500 cc. volumetric flask and carefully dry the neck of the flask. Weigh about 5 grams of the sample in a weighing bottle and transfer to the flask with the least possible exposure to the air. Wash down into the flask and mix by whirling until solution is complete and the small quantity of calcium carbide has been decomposed. Then add 25 cc. of the soda-lead reagent, or a quantity sufficient to remove sulfides, close the flask with a rubber stopper, and shake thoroughly, preferably for half an hour. Dilute to the mark, mix, and filter through a dry filter. Transfer a 50 cc aliquot to a 400 cc. beaker and proceed as directed under the determination of cyanogen in sodium and potassium cyanides. One cc of 0.1 N silver nitrate solution is equivalent to 0.005202 gram of cyanogen (CN). If the percentage of calcium cyanide is desired, multiply the percentage of cyanogen by the factor 1.7702.

(6) The following methods for the determination of chlorine in calcium cyanide were adopted as official (first action).

CHLORINE.—TENTATIVE.

REAGENTS.

(The same as for chlorine in sodium and potassium cyanides.)

DETERMINATIONS.

Method I.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a beaker, and proceed as directed under Method I for the determination of chlorine in sodium and potassium cyanides

Method II.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a distilling flask, and proceed as directed under Method II for the determination of chlorine in sodium and potassium cyanides.

(7) The official method for the determination of moisture in soap (p. 65) was dropped (first action).

(8) The tentative xylene distillation method for the determination of water in soap[1] was adopted as an official method (first action).

(9) The tentative methods for the determination of (a) water, (b) total oil, and (e) ash in mineral oil-soap emulsions[2] were adopted as official methods (first action).

(10) Method I[3] for the determination of soap in mineral oil-soap emulsions, adopted at the 1925 meeting as a tentative method[4], was dropped.

(11) The tentative method for the determination of soap in mineral oil-soap emulsions[5], with the statement "In this method error will result if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid" appended, was adopted as an official method (first action).

(12) The following method for the determination of unsulfonated residue in mineral oils was adopted as an official method (first action).

MINERAL OILS.

UNSULFONATED RESIDUE.—TENTATIVE.

REAGENTS.

38 N sulfuric acid.—Prepared as directed on p. 408, 83.

DETERMINATION.

With a pipet measure 5 cc. of the oil into a Babcock cream bottle about 15 cm. (6 inches) long, either the 9 gram 50 per cent or the 18 gram 30 per cent type. (With heavy oils, to reduce the viscosity, warm the pipet after a preliminary draining by drawing it several times through the flame of a Bunsen burner and then drain thoroughly.) In lieu of measuring, determine the specific gravity of the oil and weigh the equivalent of 5 cc. into the bottle. Add slowly 20 cc. of the 38 *N* sulfuric acid, gently shaking or rotating the bottle and taking care that the temperature does not rise above 60°C. Cool in ice water if necessary. When the mixture no longer develops heat on shaking, agitate thoroughly, place the bottle in a water bath, and heat at 60°–65°C. for 10 minutes, keeping the contents of the bottle thoroughly mixed by shaking vigorously for a period of 20 seconds at 2 minute intervals. Remove the bottle from the bath and fill with concentrated sulfuric acid until the oil rises into the graduated neck. Centrifugalize for 5 minutes (or longer if necessary to obtain a constant volume of oil) at 1200–1500 revolutions per minute. Read the volume of unsulfonated residue from the graduations on the neck of the bottle and, to convert to cubic centimeters, multiply the reading from the 9 gram 50 per cent bottle by 0.1 and that from the 18 gram 30 per cent bottle by 0.2. From the result thus obtained calculate the percentage by volume of the unsulfonated oil.

(13) The tentative method for the determination of unsulfonated residue in mineral oil-soap emulsions[5] was deleted, the method for the

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[1] *This Journal*, 1926, 9: 27.
[2] *Ibid.*, 28, 29.
[3] *Ibid.*, 129.
[4] *Ibid.*, 71.
[5] *Ibid.*, 28.

determination of unsulfonated residue in mineral oils was substituted therefor under the title "Unsulfonated Residue of Recovered Oil," and the method then made official (first action) for the determination of the unsulfonated residue of the oil recovered from mineral oil-soap emulsions.

VI. TANNING MATERIALS.

No additions, deletions, or other changes.

VII. LEATHERS.

(1) The tentative method for the determination of moisture in vegetable tanned leather (p. 79) was dropped.

(2) The toluene distillation method was adopted as a tentative method for the determination of moisture in vegetable tanned leather. The method is as follows:

MOISTURE.

By Toluene Distillation.—Tentative.

APPARATUS.

(a) *500 cc. flask.*—Erlenmeyer or distilling flask of Pyrex or other resistant glass.

(b) *Receiving tube.*—Graduated in tenths of a cubic centimeter.

(c) *Liebig condenser.*—Sealed-in, straight-tube, about 25 cm. (10 inches) long, with delivery tube approximately 9.5 mm. (0.375 inch) in diameter.

Assemble the apparatus as shown in the figure[1]. Before each distillation clean the condenser and receiving tube with chromic-sulfuric acid mixture, rinse thoroughly with water, then with alcohol, and dry either in an oven or with a current of air. Calibrate the receiving tube by distilling toluene containing known quantities of water. Read the bottom of the meniscus of the water column and estimate as closely as possible to hundredths of a cubic centimeter.

DETERMINATION.

Weigh 20 grams of the prepared sample and transfer to the distilling flask. Immediately add about 200 cc. of dry toluene having a boiling point, under normal pressure, of 110° to 112°C., and connect the flask with the receiving tube and condenser. Fill the receiving tube with toluene, pouring it through the condenser. Heat the distilling flask gently and distil at the rate of about 4 drops per second for exactly 2 hours. At the end of 1, 1.25, 1.5, 1.75, and 2 hours' distillation, wash down the condenser by pouring toluene in at the top while brushing thoroughly with a tight-haired, close fitting tube brush that has been boiled previously in toluene. (A long handle may be made by fastening to the brush a piece of heavy copper wire.) At the end of 2 hours disconnect the receiving tube, dislodge any drops of water on the inside by rubbing with a piece of light copper wire twisted at one end into a loop, and allow the tube to come to room temperature. Read the volume of water as accurately as possible to 0.01 cc. and make such calibration correction as may be necessary. Assuming that 1 cc. of water weighs 1 gram, calculate the percentage of moisture.

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[1] *This Journal,* 1926, **9**: 30.

VIII. WATERS, BRINE, AND SALT.

To correct a typographical error in the method for the determination of manganese by the bismuthate method (p. 101), the word "bisulfate" in lines 8 and 17 of paragraph 75 was changed to bisulfite.

IX. FEEDING STUFFS.

(1) The method for the determination of the oat hulls in ground oats[1], but somewhat revised, was adopted as a tentative method for the determination of oat hulls in oat feeds. The method adopted is as follows:

OAT HULLS IN OATS AND OAT FEEDS.—TENTATIVE.

(Results are approximate only.)

Place in a 1000 cc. beaker 800 cc. of water and 2 grams of the sample, previously ground to pass through a sieve having circular openings 1 mm. in diameter. Stir vigorously to obtain a centrifugal effect, allow to stand for 5 minutes, and then decant the supernatant liquid carefully, retaining so far as possible all hull particles. Repeat this procedure several times until the supernatant liquid becomes clear, or nearly so, and then transfer the residue with the aid of 150 cc. of water to a 300 cc. beaker. Add 5 drops of strong hydrochloric acid and boil for 2 minutes, constantly stirring the mixture. Transfer to the original beaker with the aid of 500 cc. of water, stir, and allow to stand until the supernatant liquid is clear. Draw off the liquid by means of a siphon of rubber tubing having a 3 or 4 mm. bore, using a pinch clamp to control the flow so that practically all the liquid may be siphoned off. (Tilting the beaker will also help to obtain this result.) If on standing a deposit forms in the siphonate, repeat the siphonation. Transfer the hulls with the aid of water to a paper filter, wash several times with alcohol, and allow to dry to constant weight at room temperature. When dry, carefully remove the hulls from the paper, using if necessary a small stiff brush, and weigh. (A weighed Gooch crucible may be used instead of the paper filter.) Multiply the weight of hulls by 50 to obtain the percentage of hulls in the sample.

(2) The tentative distillation method for the determination of moisture[2] was adopted as an official method (first action).

X. PRESERVATIVES AND ARTIFICIAL SWEETENERS.

No additions, deletions, or other changes.

XI. COLORING MATTERS IN FOODS.

No additions, deletions, or other changes.

XII. METALS IN FOODS.

No additions, deletions, or other changes.

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[1] *This Journal*, 1926, 9 149.

[2] *Ibid.*, 30.

XIII. SUGARS AND SUGAR PRODUCTS.

No additions, deletions, or other changes.

XIV. FRUITS AND FRUIT PRODUCTS.

No additions, deletions, or other changes.

XV. CANNED VEGETABLES.

No additions, deletions, or other changes.

XVI. CEREAL FOODS.

(1) The official vacuum method for the determination of moisture in flour (p. 225, **1**) was dropped (first action).

(2) The vacuum oven method for the determination of total solids and, indirectly, of moisture in flour[1] was made official (final action).

(3) The tentative routine air-oven method for the determination of total solids and, indirectly, of moisture in flour[2], but with the word "routine" deleted from the title, was adopted as official (first action).

(4) The method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour[2] was made official (final action).

(5) The method for the determination of lipoids and lipoid phosphoric acid (P_2O_5) in flour[2] was made official (final action).

(6) The tentative acid hydrolysis method for the determination of fat in flour[3] was adopted as an official method (first action).

(7) The modified Kerr-Sorber method[4] was adopted as a tentative method for the determination of the unsaponifiable matter in the fat of flour. However, instead of 5 grams of fat, the extract obtained from 5 grams of flour as directed under Lipoids should be used for the determination.

(8) The following method for the determination of the hydrogen-ion concentration of flour was adopted as a tentative method.

HYDROGEN-ION CONCENTRATION.—TENTATIVE.

Weigh 10 grams of the flour (or some multiple thereof) into a clean, dry Erlenmeyer flask and add for each 10 grams of flour 100 cc. of distilled water at a temperature of 25°C. Shake or whirl the flask until the particles of flour are evenly suspended and the mixture is free from lumps. Place in a thermostat at 25°C. and shake, continuously or intermittently in such a manner as to keep the flour particles in suspension, for 30 minutes. Let stand quietly for 10 minutes, then decant the supernatant liquid into a suitable vessel, and immediately determine its hydrogen-ion concentration electrometrically.

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[1] *This Journal*, 1926, **9**: 39.
[2] *Ibid.*, 40.
[3] *Ibid.*, 41.
[4] *Ibid.*, 1925, **8**: 441.

(9) The method for the determination of ash in baked cereal products[1] was made official (final action).

(10) The method for the determination of protein in baked cereal products[1] was made official (final action).

(11) The method for the determination of ash in alimentary pastes (p. 232) was made official (final action).

(12) The method under alimentary pastes for the determination of chlorides in ash as sodium chloride (p. 232) was made official (final action).

(13) The method for the determination of organic and ammoniacal nitrogen in alimentary pastes (p. 232) was made official (final action).

(14) The method for the determination of protein in alimentary pastes[2] was made official (final action).

(15) The method for the extraction and identification of added color in alimentary pastes (p. 233) was made official (final action).

(16) The acid hydrolysis method for the determination of fat in flour[3] was adopted as a tentative method for the determination of fat in alimentary pastes.

(17) The modified Kerr-Sorber method, with the procedure given under (7) above, was adopted as a tentative method for the determination of the unsaponifiable matter in the fat of alimentary pastes.

XVII. MEAT AND MEAT PRODUCTS.

No additions, deletions, or other changes.

XVIII. GELATIN.

No additions, deletions, or other changes.

XIX. DAIRY PRODUCTS.

(1) The cryoscopic method for the detection and determination of added water in milk (p. 265) was adopted as an official method (first action) for the detection and determination of added water in cream, but when used on cream the percentage of added water is to be found as follows:

Ascertain the percentage of added water by means of the formula—

$$W = \frac{\%\ \text{Serum in Cream}\ (T - T')}{T}, \text{ in which}$$

W = the percentage of added water;
T = the freezing point of undiluted cream (-0.550°C.);
T' = the observed freezing point of the given sample; and
% Serum = 100% − (% fat + % protein).

If protein is not determined it may be assumed to be 38 per cent of the solids-not-fat.

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[1] *This Journal*, 1926, 9: 42.
[2] *Ibid.*, 44.
[3] *Ibid.*, 41.

(2) The method for the determination of moisture in cheese[1] was made official (final action).

(3) The method for the preparation of sample, as described for malted milk (p. 275, **61**), was adopted as tentative for dried milk.

(4) The methods for the determination of protein and of ash, as described for malted milk (p. 275, **63** and **64**), were adopted as tentative for dried milk.

XX. FATS AND OILS.

(1) The official method for the determination of unsaponifiable residue in fats and oils (p. 295) was dropped (final action).

(2) The F. A. C. method for the determination of unsaponifiable matter, as printed in *This Journal*, 1926, **9**: 45, but with the sentence "cool the cylinder and contents to room temperature and add 50 cc. of the petroleum ether" in the sixth and seventh lines under the heading "Determination" changed to "Rinse the flask with 50 cc. of petroleum ether and add the rinsings to the contents of the cylinder previously cooled to room temperature", was made official (final action).

(3) The André-Cook method for the determination of acetyl value, slightly modified in technique, was adopted as official (first action), The method, as adopted, is as follows:

ACETYL VALUE.—TENTATIVE.

Acetylation.

Boil 50 cc. of the sample with 50 cc. of freshly distilled acetic anhydride under a reflux condenser for 2 hours. Pour the mixture into 500 cc. of water in a beaker and boil for 15 minutes while bubbling a stream of air or of carbon dioxide through the solution to prevent bumping. Siphon off the water, add 500 cc. more of water, and boil again for 15 minutes. Repeat the siphonation and boil for 15 minutes with a third 500 cc. portion of water. Allow the mixture to cool and separate the aqueous layer, which should be neutral to litmus. Transfer the acetylated oil to a separatory funnel and wash with two 200 cc. portions of warm water. Separate as much of the water as possible, add 5 grams of anhydrous sodium sulfate to the acetylated oil, and let stand for 1 hour, agitating occasionally to assist the drying. Filter through a dry folded filter, preferably in an oven heated to 100°-110°C., and keep the filtered oil in the oven until the oil is completely dry. The acetylated product should be a clear, brilliant oil.

Saponification.

Weigh accurately about 2 to 2.5 grams each of the acetylated oil and of the untreated oil into separate 250 cc. Erlenmeyer flasks. Add to each flask exactly 25 cc. of alcoholic potassium hydroxide solution, prepared as directed on p. 288, **21** (**b**), and reflux for 1 hour. Titrate the warm solutions with 0.5 *N* hydrochloric acid, using phenolphthalein as indicator. Titrate in the same way two 25 cc. portions of the alcoholic potassium hydroxide solution. From the mean of these two results, which should be in very close agreement, deduct the volume of the standard hydrochloric acid required for the titration of the acetylated and of the untreated oil and from the results so obtained calculate the saponification number (mg. of potassium hydroxide required to saponify 1 gram of product) of each. Calculate the acetyl value by means of the following formula:

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[1] *This Journal*, 1926, **9**: 44.

$$A = \frac{S' - S}{1\text{–}0.00075S}, \text{ in which}$$

A = acetyl value;
S = saponification number of oil; and
S' = saponification number of the acetylated oil.

(4) The official method for the determination of acetyl value (p. 293) was dropped (first action).

XXI. BAKING POWDERS AND BAKING CHEMICALS.

(1) The electrolytic method for the determination of lead (p. 310, **30**, **31**, and **32**) was made official (final action).

(2) In the tentative gasometric method for the determination of total carbon dioxide (p. 305, **8**, **9**, and **10**) paragraph **10** was deleted and the following paragraph was substituted therefor:

10 DETERMINATION.

Weigh 1.7 grams of the sample, prepared as directed under **1**, into a dry decomposition flask (*A*) and connect this flask with the apparatus (Fig. 21). Open the T-tube stopcock (*C*) and by means of the leveling bulb (*E*) bring the displacement solution to the 10 cc. graduation above the zero mark. (This 10 cc. is equal in volume to the volume of acid to be used in the decomposition.) Allow the apparatus to stand 1 to 2 minutes to insure equalization of temperature and pressure within the apparatus with that of the room. Close the stopcock, lower the leveling bulb somewhat to reduce the pressure within the apparatus, and slowly run into the decomposition flask from the buret (*F*) 10 cc. of the dilute sulfuric acid. To prevent the liberated carbon dioxide from escaping through the acid buret into the air, keep the displacement solution in the leveling bulb at all times during the decomposition at a lower level than that in the gas-measuring tube. Rotate and then vigorously agitate the decomposition flask to secure intimate mixture of the contents. Allow to stand for 5 minutes to secure equilibrium. Equalize the pressure in the measuring tube by means of the leveling bulb and read the volume of gas in the tube. Observe the temperature of the air surrounding the apparatus and also the barometric pressure obtaining at the time and multiply the number of cubic centimeters of gas evolved by the factor given in the table for this temperature and pressure. Divide the corrected reading by 10 to obtain the percentage by weight of carbon dioxide in the sample.

The method, as amended, was adopted as official (first action).

(3) The tentative gasometric method for the determination of residual carbon dioxide (p. 306, **12**) was amended by striking out the words "the factor weight" in the first line and substituting "1.7 grams" therefor. The method, as amended, was adopted as official (first action).

Correction factors for the gasometric determination of carbon dioxide.

Based on Sample Weighing 1.7000 Grams.[1]

°C.	15 0°	15 5°	16 0°	16 5°	17.0°	17.5°	18.0°	18.5°	
mm.									*inches.*
700	0.99194	0.99006	0.98818	0.98573	0.98329	0.98082	0.97835	0.97585	27.56
702	0.99494	0.99300	0.99106	0.98862	0.98618	0.98368	0.98118	0.97868	27.64
704	0.99794	0.99544	0.99394	0.99147	0.98900	0.98653	0.98406	0.98156	27.72
706	1.00094	0.99886	0.99682	0.99435	0.99188	0.98941	0.98694	0.98406	27.80
708	1.00394	1.00183	0 99971	0.99723	0.99476	0.99226	0.98976	0.98726	27.87
710	1.00694	1.00477	1.00259	1.00012	0.99765	0.99512	0.99259	0.99009	27.95
712	1,00994	1.00767	1.00541	1.00294	1.00047	0.99795	0.99541	0.99291	28.03
714	1.01294	1.01061	1.00829	1.00582	1.00335	1.00080	0.99824	0.99576	28.11
716	1.01594	1.01356	1.01118	1.00871	1.00624	1.00368	1.00112	0.99861	28.19
718	1.01894	1.01650	1.01406	1.01156	1.00906	1.00653	1.00400	1.00150	28.27
720	1 02194	1.01949	1.01694	1.01444	1.01194	1.00941	1.00688	1.00435	28.35
722	1.02482	1.02232	1.01982	1.01732	1.01482	1.01229	1.00976	1.00720	28.43
724	1.02771	1.02521	1.02271	1.02021	1.01771	1.01518	1.01265	1 01009	28.50
726	1.03059	1.02809	1 02559	1 02306	1.02053	1.01800	1.01547	1.01291	28.58
728	1.03347	1.03097	1.02847	1.02594	1.02341	1.02088	1 01835	1.01580	28.66
730	1.03635	1.03385	1.03135	1.02882	1.02629	1.02374	1.02118	1.01862	28.74
732	1.03924	1.03674	1 03424	1.03171	1 02918	1.02662	1.02406	1.02147	28 82
734	1.04218	1.03915	1.03712	1.03459	1.03206	1.02950	1.02694	1.02435	28.90
736	1.04506	1.04253	1.04000	1.03744	1 03488	1 03232	1.02976	1.02718	28.98
738	1.04794	1.04541	1.04288	1.04037	1.03776	1.03521	1.03265	1.03006	29.06
740	1.05082	1.04829	1.04576	1 04321	1.04065	1.03806	1.03547	1.03288	29.13
742	1.05371	1.05118	1.04865	1.04609	1.04353	1.04094	1.03835	1 03577	29.21
744	1.05659	1.05403	1 05147	1.04991	1.04635	1.04377	1.04118	1.03859	29.29
746	1.05947	1.05691	1.05435	1.05180	1.04924	1.04665	1.04406	1.04147	29.37
748	1.06235	1.05929	1.05724	1.05418	1.05212	1.04953	1.04694	1.04433	29.45
750	1.06524	1.06218	1.06012	1.05748	1.05494	1.05235	1.04976	1.04715	29.53
752	1.06818	1.06512	1.06306	1.06047	1.05788	1.05527	1.05265	1.05003	29.61
754	1.07106	1.06847	1.06588	1.06330	1.06071	1.05812	1.05553	1.05289	29.69
756	1.07394	1.07135	1.06876	1.06618	1.06359	1.06197	1.05835	1 05571	29.76
758	1.07682	1.07423	1.07165	1.06906	1.06647	1.06386	1.06124	1.05859	29.84
760	1.07971	1.07712	1.07453	1.07191	1.06929	1.06668	1.06406	1.06141	29.92
762	1.08259	1.08050	1.07741	1.07480	1.07218	1.06956	1.06694	1.06430	30.00
764	1.08547	1.08288	1.08029	1.07768	1.07506	1.07244	1.06982	1.06715	30.08
766	1.08841	1.08580	1.08318	1.08056	1.07794	1.07530	1.07265	1.06997	30.16
768	1.09129	1.08868	1.08606	1.08344	1.08082	1.07818	1.07553	1.07285	30.24
770	1.09418	1.09156	1.08894	1.08630	1.08365	1.08100	1.07835	1.07567	30.31
°F.	59.0°	59.9°	60 8°	61 7°	62 6°	63 5°	64 4°	65 3°	

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[1] Calculated from 1.976 = weight of one liter CO_2 at 0° C., 760 mm pressure and 41° latitude Formula given by S. W. Parr, *J. Am. Chem. Soc.*, 1909, **31**: 237.

Correction factors for the gasometric determination of carbon dioxide.—Continued.

°C.	19.0°	19.5°	20 0°	20 5°	21 0°	21 5°	22 0°	22 5°	
mm.									*inches*
700	0.97335	0.97085	0.96835	0.96564	0.96294	0.96023	0.95753	0.95509	27.56
702	0.97618	0.97368	0.97118	0.96850	0.96582	0.96311	0 96041	0.95794	27.64
704	0.97906	0.97653	0.97400	0.97132	0.96865	0.96597	0.96329	0.96082	27.72
706	0.98188	0.97938	0.97688	0.97420	0.97153	0.96888	0.96624	0.96371	27.80
708	0.98476	0.98224	0.97971	0.97703	0.97435	0.97173	0 96912	0.96656	27.87
710	0.98759	0.98506	0.98253	0.97988	0.97724	0.97459	0.97195	0.96938	27.95
712	0.99041	0.98788	0.98535	0.98273	0.98012	0.97747	0.97483	0.97227	28.03
714	0.99329	0.99073	0.98818	0.98556	0.98294	0.98032	0.97771	0.97512	28.11
716	0.99612	0.99358	0.99106	0.98844	0.98582	0.98323	0.98065	0.97800	28.19
718	0.99900	0.99644	0.99388	0.99126	0.98865	0.98606	0.98348	0.98083	28.27
720	1.00182	0.99925	0.99671	0.99412	0.99153	0.98894	0.98636	0.98371	28.35
722	1.00465	1.00209	0.99953	0.99694	0.99435	0.99176	0.98918	0.98653	28.43
724	1.00753	1.00497	1.00241	0.99982	0.99724	0.99462	0.99200	0.98932	28.50
726	1.01035	1.00779	1.00524	1.00265	1.00006	0.99746	0.99483	0.99215	28.58
728	1.01324	1.01065	1.00806	1.00547	1.00288	1.00027	0.99765	0.99497	28.66
730	1.01606	1.01347	1.01088	1.00829	1.00571	1.00306	1.00041	0.99781	28.74
732	1.01888	1.01629	1.01371	1.01112	1.00853	1.00588	1.00324	1.00056	28.82
734	1.02176	1.01919	1.01659	1.01497	1.01135	1.00870	1.00606	1.00338	28.90
736	1.02459	1.02200	1.01941	1.01679	1.01418	1.01153	1.00888	1.00620	28.98
738	1.02747	1.02486	1.02224	1.01962	1.01700	1.01435	1.01171	1.00900	29.06
740	1.03029	1.02768	1,02506	1.02244	1.01982	1.01717	1.01453	1.01182	29.13
742	1.03318	1.03056	1.02794	1.02529	1.02265	1.02000	1.01735	1.01464	29.21
744	1.03600	1.03338	1.03076	1.02811	1.02547	1.02279	1.02212	1.01752	29.29
746	1.03888	1.03624	1.03359	1.03094	1.02829	1.02561	1.02294	1.02024	29.37
748	1.04171	1.03906	1.03641	1.03376	1.03112	1.02844	1.02576	1.02306	29.45
750	1.04453	1.04189	1.03924	1.03659	1.03394	1.03126	1.02859	1.02589	29.53
752	1.04741	1.04477	1.04212	1.03944	1.03676	1.03408	1.03141	1.02868	29.61
754	1.05024	1.04759	1.04494	1.04226	1.03959	1.03691	1.03424	1.03150	29.69
756	1.05306	1.05041	1.04776	1.04508	1.04241	1.03973	1.03706	1.03433	29.76
758	1.05594	1.05330	1.05065	1.04797	1.04529	1.04259	1.03988	1.03715	29.84
760	1.05876	1.05612	1.05347	1.05079	1.04812	1.04539	1.04265	1.03992	29.92
762	1.06165	1.05897	1.05629	1.05361	1.05094	1.04821	1.04547	1.04274	30.00
764	1.06447	1.06179	1.05912	1.05644	1.05376	1.05103	1.04829	1.04556	30.08
766	1.06729	1.06462	1.06194	1.05926	1.05659	1.05386	1.05112	1.04839	30.16
768	1.07018	1.06750	1.06482	1.06212	1.05941	1.05668	1.05394	1.05118	30.24
770	1.07300	1,07032	1.06765	1.06424	1.06224	1.05950	1.05676	1.05400	30.31
°F.	66.2°	67.1°	68 0°	68 9°	69 8°	70 7°	71.6°	72.5°	

Correction factors for the gasometric determination of carbon dioxide—Continued.

°C	23.0°	23.5°	24.0°	24.5°	25.0°	25.5°	26.0°	26.5°	
mm									*inches*
700	0.95265	0.95020	0.94776	0.94508	0.94241	0.93973	0.93706	0.93432	27.56
702	0.95547	0.95303	0.95059	0.94788	0.94518	0.94250	0.93982	0.93708	27.64
704	0.95835	0.95585	0.95335	0.95067	0.94800	0.94532	0.94265	0.93988	27.72
706	0.96118	0.95865	0.95612	0.95344	0.95076	0.94808	0.94541	0.94267	27.80
708	0.96400	0.96147	0.95894	0.95626	0.95359	0.95088	0.94818	0.94544	27.87
710	0.96682	0.96429	0.96176	0.95905	0.95635	0.95364	0.95094	0.94820	27.95
712	0.96971	0.96712	0.96453	0.96182	0.95912	0.95644	0.95376	0.95100	28.03
714	0.97253	0.96991	0.96729	0.96461	0.96194	0.95923	0.95653	0.95376	28.11
716	0.97535	0.97273	0.97012	0.96741	0.96471	0.96200	0.95929	0.95655	28.19
718	0.97818	0.97556	0.97294	0.97023	0.96753	0.96482	0.96212	0.95935	28.27
720	0.98106	0.97838	0.97571	0.97300	0.97029	0.96758	0.96488	0.96213	28.35
722	0.98388	0.98120	0.97853	0.97582	0.97312	0.97038	0.96765	0.96488	28.43
724	0.98665	0.98397	0.98129	0.97858	0.97588	0.97314	0.97041	0.96764	28.50
726	0.98947	0.98679	0.98412	0.98141	0.97871	0.97594	0.97318	0.97041	28.58
728	0.99229	0.98961	0.98694	0.98420	0.98147	0.97870	0.97594	0.97319	28.66
730	0.99512	0.99241	0.98971	0.98697	0.98424	0.98147	0.97871	0.97594	28.74
732	0.99788	0.99517	0.99247	0.98973	0.98700	0.98423	0.98147	0.97871	28.82
734	1.00071	0.99799	0.99529	0.99255	0.98982	0.98705	0.98429	0.98165	28.90
736	1.00353	1.00083	0.99812	0.99538	0.99265	0.98985	0.98706	0.98426	28.98
738	1.00629	1.00359	1.00088	0.99815	0.99541	0.99261	0.98982	0.98703	29.06
740	1.00912	1.00643	1.00371	1.00095	1.99818	0.99538	0.99259	0.98976	29.13
742	1.01194	1.00923	1.00653	1.00377	1.00100	0.99820	0.99541	0.99258	29.21
744	1.01471	1.01200	1.00929	1.00643	1.00376	1.00097	0.99818	0.99535	29.29
746	1.01753	1.01482	1.01212	1.00936	1.00659	1.00376	1.00094	0.99809	29.37
748	1.02035	1.01762	1.01488	1.01212	1.00935	1.00653	1.00371	1.00088	29.45
750	1.02318	1.02045	1.01771	1.01492	1.01212	1.00936	1.00659	1.00370	29.53
752	1.02594	1.02321	1.02047	1.01771	1.01494	1.01211	1.00929	1.00644	29.61
754	1.02876	1.02603	1.02329	1.02050	1.01771	1.01483	1.01206	1.00921	29.69
756	1.03159	1.02883	1.02606	1.02326	1.02047	1.01764	1.01482	1.01197	29.76
758	1.03441	1.03165	1.02888	1.02608	1.02329	1.02047	1.01765	1.01477	29.84
760	1.03718	1.03442	1.03165	1.02886	1.02606	1.02323	1.02041	1.01753	29.92
762	1.04000	1.03724	1.03447	1.03164	1.02882	1.02600	1.02318	1.02030	30.00
764	1.04282	1.04003	1.03723	1.03444	1.03165	1.02880	1.02594	1.02306	30.08
766	1.04565	1.04285	1.04005	1.03723	1.03441	1.03156	1.02871	1.02583	30.16
768	1.04841	1.04562	1.04282	1.04003	1.03724	1.03435	1.03147	1.02859	30.24
770	1.05124	1.04844	1.04564	1.04282	1.04000	1.03712	1.03424	1.03136	30.31
°F.	73.4°	74.3°	75.2°	76.1°	77.0°	77.9°	78.8°	79.7°	

Correction factors for the gasometric determination of carbon dioxide.—Continued.

°C.	27 0°	27.5°	28 0°	28 5°	29.0°	29.5°	30 0°	30.5°	
mm.									*inches*
700	0.93159	0.92885	0.92612	0.92332	0.92053	0.91773	0.91494	0.91203	27.56
702	0.93435	0.93161	0.92888	0.92608	0.92329	0.92047	0.91765	0.91476	27.64
704	0.93712	0.93438	0.93165	0.92882	0.92600	0.92320	0.92041	0.91750	27.72
706	0.93994	0.93717	0.93441	0.93158	0.92876	0.92594	0.92312	0.92024	27.80
708	0.94271	0.93994	0.93718	0.93435	0.93153	0.92870	0.92588	0.92297	27.87
710	0.94547	0.94267	0.93988	0.93706	0.93424	0.93141	0.92859	0.92567	27.95
712	0.94824	0.94544	0.94265	0.93982	0.93700	0.93414	0.93129	0.92841	28.03
714	0.95100	0.94820	0.94541	0.94258	0.93976	0.93691	0.93406	0.93115	28.11
716	0.95382	0.95100	0.94818	0.94535	0.94253	0.93964	0.93676	0.93388	28.19
718	0.95659	0.95376	0.95094	0.94809	0.94524	0.94238	0.93953	0.93662	28.27
720	0.95939	0.95655	0.95371	0.95085	0.94800	0.94512	0.94224	0.93932	28.35
722	0.96212	0.95929	0.95647	0.95361	0.95076	0.94788	0.94500	0.94209	28.43
724	0.96488	0.96206	0.95924	0.95638	0.95353	0.95062	0.94771	0.94479	28.50
726	0.96765	0.96482	0.96200	0.95912	0.95624	0.95332	0.95041	0.94750	28.58
728	0.97041	0.96758	0.96476	0.96188	0.95900	0.95609	0.95318	0.95026	28.66
730	0.97318	0.97036	0.96753	0.96464	0.96176	0.95885	0.95594	0.95300	28.74
732	0.97594	0.97309	0.97024	0.96735	0.96447	0.96156	0.95865	0.95578	28.82
734	0.97871	0.97585	0.97300	0.97012	0.96724	0.96429	0.96135	0.95844	28.90
736	0.98147	0.97861	0.97576	0.97288	0.97000	0.96706	0.96412	0.96118	28.98
738	0.98424	0.98138	0.97853	0.97564	0.97276	0.96982	0.96688	0.96394	29.06
740	0.98694	0.98409	0.98124	0.97835	0.97547	0.97253	0.96959	0.96665	29.13
742	0.98976	0.98691	0.98406	0.98115	0.97824	0.97529	0.97235	0.96941	29.21
744	0.99253	0.98967	0.98682	0.98391	0.98100	0.97806	0.97512	0.97215	29.29
746	0.99529	0.99241	0.98953	0.98662	0.98371	0.98076	0.97782	0.97485	29.37
748	0.99806	0.99517	0.99229	0.98938	0.98647	0.98353	0.98059	0.97762	29.45
750	1.00082	0.99796	0.99506	0.99215	0.98924	0.98626	0.98329	0.98032	29.53
752	1.00359	1.00071	0.99782	0.99491	0.99200	0.98903	0.98606	0.98306	29.61
754	1.00635	1.00342	1.00059	0.99738	0.99471	0.99173	0.98876	0.98579	29.69
756	1.00912	1.00624	1.00335	1.00041	0.99747	0.99450	0.99153	0.98853	29.76
758	1.01188	1.00900	1.00612	1.00318	1.00024	0.99724	0.99429	0.99129	29.84
760	1.01465	1.01174	1.00882	1.00588	1.00294	0.99995	0.99700	0.99400	29.92
762	1.01741	1.01450	1.01159	1.00865	1.00571	1.00274	0.99976	0.99673	30.00
764	1.02018	1.01727	1.01435	1.01141	1.00847	1.00547	1.00247	0.99948	30.08
766	1.02294	1.02003	1.01712	1.01418	1.01124	1.00824	1.00524	1.00221	30.16
768	1.02571	1.02280	1.01988	1.01611	1.01394	1.01094	1.00794	1.00491	30.24
770	1.02847	1.02556	1.02265	1.01968	1.01671	1.01371	1.01071	1.00768	30.31
°F.	80.6°	81 5°	82 4 °	83.3°	84.2°	85 1°	86.0°	86.9°	

Correction factors for the gasometric determination of carbon dioxide.—Concluded.

°C.	31.0°	31.5°	32.0°	32.5°	33.0°	33.5°	34.0°	34.5°	35.0°	
mm										*inches*
700	0.90912	0.90620	0.90329	0.90082	0.89735	0.89432	0.89129	0.88821	0.88512	27.56
702	0.91188	0.90894	0.90600	0.90303	0.90006	0.89703	0.89400	0.89091	0.88782	27.64
704	0.91459	0.91165	0.90871	0.90576	0.90282	0.89976	0.89671	0.89362	0.89053	27.72
706	0.91735	0.91441	0.91147	0.90847	0.90547	0.90241	0.89935	0.89627	0.89318	27.80
708	0.92006	0.91712	0.91418	0.91118	0.90818	0.90512	0.90206	0.89897	0.89588	27.87
710	0.92276	0.91982	0.91688	0.91388	0.91088	0.90782	0.90476	0.90168	0.89859	27.95
712	0.92553	0.92256	0.91959	0.91659	0.91359	0.91053	0.90747	0.90438	0.90129	28.03
714	0.92824	0.92529	0.92235	0.91932	0.91629	0.91323	0.91018	0.90706	0.90394	28.11
716	0.93100	0.92803	0.92506	0.92203	0.91900	0.91594	0.91288	0.90976	0.90665	28.19
718	0.93371	0.93078	0.92776	0.92474	0.92171	0.91865	0.91559	0.91247	0.90935	28.27
720	0.93641	0.93344	0.93047	0.92744	0.92441	0.92135	0.91829	0.91517	0.91206	28.35
722	0.93918	0.93618	0.93318	0.93015	0.92712	0.92412	0.92100	0.91785	0.91471	28.43
724	0.94188	0.93897	0.93606	0.93294	0.92982	0.92676	0.92371	0.92056	0.91741	28.50
726	0.94459	0.94159	0.93859	0.93556	0.93253	0.92944	0.92635	0.92323	0.92012	28.58
728	0.94735	0.94435	0.94135	0.93830	0.93544	0.93215	0.92906	0.92591	0.92276	28.66
730	0.95006	0.94706	0.94406	0.94103	0.93800	0.93488	0.93176	0.92861	0.92547	28.74
732	0.95282	0.94979	0.94676	0.94373	0.94071	0.93759	0.93447	0.93132	0.92818	28.82
734	0.95553	0.95250	0.94947	0.94644	0.94341	0.94034	0.93718	0.93403	0.93088	28.90
736	0.95824	0.95521	0.95218	0.94915	0.94612	0.94300	0.93988	0.93670	0.93353	28.98
738	0.96100	0.95797	0.95494	0.95188	0.94882	0.94570	0.94259	0.93941	0.93624	29.06
740	0.96371	0.96068	0.95765	0.95459	0.95153	0.94841	0.94529	0.94211	0.93894	29.13
742	0.96647	0.96341	0.96035	0.95730	0.95424	0.95112	0.94800	0.94482	0.94165	29.21
744	0.96918	0.96615	0.96312	0.96003	0.95694	0.95382	0.95071	0.94750	0.94429	29.29
746	0.97188	0.96885	0.96582	0.96273	0.95965	0.95653	0.95341	0.95020	0.94700	29.37
748	0.97465	0.97159	0.96853	0.96544	0.96235	0.95925	0.95606	0.95288	0.94971	29.45
750	0.97735	0.97429	0.97124	0.96815	0.96506	0.96191	0.95876	0.95558	0.95241	29.53
752	0.98006	0.97703	0.97400	0.97088	0.96776	0.96461	0.96147	0.95826	0.95506	29.61
754	0.98282	0.97976	0.97671	0.97359	0.97047	0.96732	0.96418	0.96097	0.95776	29.69
756	0.98553	0.98247	0.97941	0.97629	0.97318	0.97003	0.96688	0.96367	0.96047	29.76
758	0.98829	0.98521	0.98212	0.97900	0.97588	0.97273	0.96959	0.96638	0.96318	29.84
760	0.99100	0.98794	0.98488	0.98176	0.97865	0.97547	0.97229	0.96908	0.96588	29.92
762	0.99371	0.99065	0.98759	0.98443	0.98135	0.97817	0.97500	0.97176	0.96853	30.00
764	0.99647	0.99338	0.99029	0.98717	0.98406	0.98088	0.97771	0.97447	0.97124	30.08
766	0.99918	0.99609	0.99300	0.98988	0.98676	0.98356	0.98035	0.97714	0.97394	30.16
768	1.00188	0.99880	0.99571	0.99259	0.98947	0.98629	0.98312	0.97986	0.97659	30.24
770	1.00465	1.00156	0.99847	0.99532	0.99218	0.98897	0.98576	0.98252	0.97929	30.31
°F	87.8°	88.7°	89.6°	90.5°	91.4°	92.3°	93.2°	94.1°	95.0°	

XXII. SPICES AND OTHER CONDIMENTS.

No additions, deletions, or other changes.

XXIII. VINEGARS.

(1) The method for the determination of non-volatile reducing substances (p. 326) was made official (final action).

(2) The method for the determination of volatile reducing substances (p. 326) was made official (final action).

XXIV. COFFEES.

No additions, deletions, or other changes.

XXV. TEAS.

No additions, deletions, or other changes.

XXVI. CACAO PRODUCTS.

(1) The Lepper-Waterman method[1] for the determination of fat in cacao products was made official (final action).

(2) The official method (p. 345) for the determination of fat in cacao products was dropped (first action).

(3) The following method for the determination of fat in cacao products was adopted as a tentative method. (This is a modification of the old official method.)

FAT.—TENTATIVE.

REAGENTS.

(**a**) *Petroleum ether.*—Redistilled below 60°C.

(**b**) *Asbestos.*—Prepared as directed on p. 117, **15** (**c**), but further washed with alcohol, ether, and petroleum ether.

DETERMINATION.

Weigh accurately about 2 grams of the sample, prepared as directed on p. 343, **1**, and, without previous drying, stratify the charge in an extraction tube with about 0.5 gram of asbestos. Extract with petroleum ether in a continuous extractor for 4 hours. Grind the material, to break up any lumps that may have formed, and re-extract for at least 4 hours. (It is advisable to allow the solvent to run through the material once completely before applying heat for the continuous extraction.) Collect the petroleum ether extract in a weighed flask, evaporate the solvent, and dry the residue to constant weight at 100°C.

The extracted residue in the extraction tube may be used for the determination of crude fiber.

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[1] *This Journal*, 1926, **9**: 46.

XXVII. FLAVORING EXTRACTS.

(1) The Folin-Denis rapid colorimetric method for the determination of vanillin in vanilla extract and its imitations, as given in the referee's report at the 1924 meeting[1], was adopted as official (final action), under the title "VANILLIN. *Colorimetric Method.—Official*", and the title of the method for vanillin and coumarin (p. 349, 4 and 5) was changed to read "VANILLIN AND COUMARIN. *Gravimetric Method.—Official.*"

(2) The following polariscopic method for the determination of lemon oil, orange oil, and lime oil, in admixture with corn oil, cottonseed oil, peanut oil, or mineral oil, was adopted as a tentative method. The method is as follows:

OILS OF LEMON, ORANGE, AND LIMES IN VEGETABLE AND MINERAL OILS.

By Polarization—Tentative.

Polarize the sample at 20°C. in a 200 mm. tube, making five readings. From the average of these readings in degrees Ventzke subtract, for corn oil + 0.6°, for cottonseed oil − 0.3°, for peanut oil + 0.2°, and for mineral oil + 5.5°, as a correction for the rotatory effect of the menstruum. To obtain the percentage by volume of the essential oil in the mixture, divide the corrected polariscopic reading so obtained by the factor 3.4 for lemon oil in corn oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for oil of limes in corn oil, 2.3 for oil of limes in cottonseed oil, and 2.2 for oil of limes in mineral oil.

XXVIII. WINES.

No additions, deletions, or other changes.

XXIX. DISTILLED LIQUORS.

No additions, deletions, or other changes.

XXX. BEERS.

No additions, deletions, or other changes.

XXXI. DRUGS.

ACETYLSALICYLIC ACID.

(1) The tentative method for the determination of combined acetic acid[2] was made official (final action).

(2) The tentative bromine method for the determination of total salicylates (p. 388) was made official (final action).

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[1] *This Journal*, 1925, **8**: 688.
[2] *Ibid.*, 1926, **9**: 49.

(3) The tentative double titration method for the determination of acetylsalicylic acid (p. 388), as modified[1], was made official (final action). (First action was taken in 1925.)

(4) The tentative qualitative test for free salicylic acid (p. 387) was made official (final action).

ARSENICALS.

The following method for the determination of arsenic in iron-arsenic tablets was adopted as a tentative method.

ARSENIC IN IRON-ARSENIC TABLETS—TENTATIVE.

REAGENTS.

(**a**) *Fuming nitric acid.*

(**b**) *Concentrated sulfuric acid.*

(**c**) *Strong hydrochloric acid.*

(**d**) *Saturated solution of ammonium oxalate.*

(**e**) *Sodium chloride.*

(**f**) *Sodium bromide.*

(**g**) *Ferrous sulfate or hydrazine sulfate.*

(**h**) *Methyl orange indicator.*—Dissolve 1 gram of methyl orange in water and dilute to 1 liter.

(**i**) *Standard solution of potassium bromate (or of iodine).*—Standardize against pure arsenious oxide (As_2O_3). (The strength of this solution is a matter of choice. 0.5626 gram of potassium bromate dissolved in water and diluted to 1 liter will give a solution that is 0.02021 normal, 1 cc. of which is equivalent to 1 milligram of As_2O_3.)

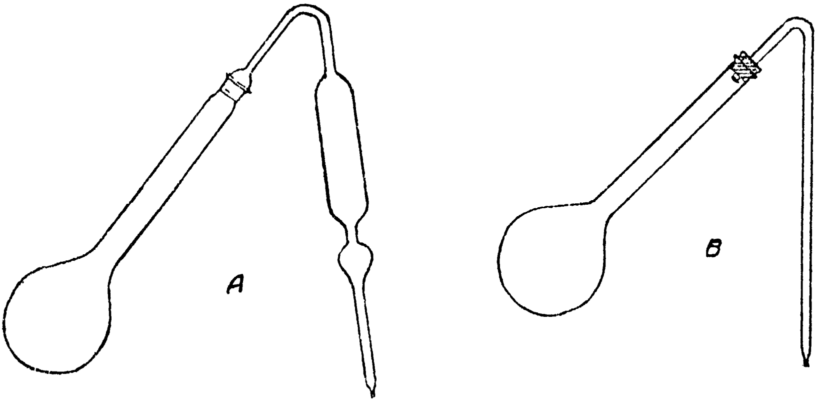

FIGURE 1.

APPARATUS.

Use either the Ramberg-Sjöström arsenic flask, which consists of a 300 cc. Kjeldahl flask provided with a specially shaped outlet tube connected with the flask by means of a ground joint (*A*, Fig. 1), or a 300 cc. Kjeldahl flask provided with an outlet tube, the internal diameter of the main part of which is about 13 mm. and that of the contracted tip about 5 mm., connected with the flask by means of a rubber stopper (*B*, Fig. 1).

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[1] *This Journal*, 1926, **9**: 49.

DETERMINATION.

Weigh and place in the flask 5–10 tablets or pills, add 10–15 cc. of water, and allow to soak for ½ hour. Then add, in small portions at a time, 20 cc. of fuming nitric acid, cooling if necessary to prevent loss by frothing. When the reaction has ceased, add carefully and in small portions at a time 25–28 cc. of concentrated sulfuric acid. Place the flask in an inclined position on an asbestos mat and heat over a small flame. As soon as the greater part of the nitric acid has been driven off and while still heating, drop in 8 cc. of fuming nitric acid through a suitably placed separatory funnel and heat over a larger flame until sulfur trioxide is evolved. If, after cooling, the sulfuric acid above the precipitated sulfates is not colorless or pale yellow, and the precipitated sulfates are not free from gray or black particles, heat the contents of the flask further with an additional 10 cc. of fuming nitric acid. (It is essential that all organic matter be destroyed.) To the cooled mixture add 30 cc. of the saturated solution of ammonium oxalate; heat until fumes of sulfur trioxide are evolved and, to insure complete destruction of the oxalic acid, for 10 minutes thereafter, over a low flame; cool; and add while gently whirling the flask 20 cc. of water. Dry the neck of the flask over a small flame and add 30 grams of sodium chloride, 5 grams of ferrous sulfate (or 1 gram of hydrazine sulfate), 1 gram of sodium bromide, and 25 cc. of strong hydrochloric acid. Mix the contents of the flask and connect the delivery tube. If the Ramberg-Sjöström apparatus is used, moisten the ground-glass joint with a drop of concentrated sulfuric acid. Fix the flask in an inclined position with the tip of the outlet tube about 1 cm. under the surface of 150 cc. of water in an Erlenmeyer flask surrounded by ice or by cold water. Distil at such a rate that the bend at the top of the tube becomes warm in 4 minutes and the lower end in about 8 minutes from the time the heat is applied. Discontinue the distillation at the end of 10 minutes, but before removing the flame lift the distillation flask until the tip of the outlet tube is above the water in the receiving flask. Let the outlet tube drain, remove the receiver, and either titrate with the standard solution of potassium bromate, using 2 drops of methyl orange indicator (the red color of the indicator at the end point may fade slowly, but the color should persist for at least 1 minute upon addition of another drop of the indicator), or nearly neutralize with sodium hydroxide, add 4–5 grams of sodium bicarbonate, and titrate with the standard solution of iodine, using starch indicator.

CAMPHOR AND MONOBROMATED CAMPHOR.

Method I for the determination of monobromated camphor in tablets (p. 393) was made official (final action).

CHLOROFORM AND CARBON TETRACHLORIDE.

The following method for the determination of chloroform or of carbon tetrachloride was adopted as a tentative method.

CHLOROFORM AND CARBON TETRACHLORIDE.—TENTATIVE.

REAGENTS.

(a) *Alcoholic potassium hydroxide.*—Dissolve 30 grams of potassium hydroxide in 30 cc. of water. Cool, and dilute to 100 cc. with methyl alcohol.

(b) *0.1 N silver nitrate solution.*

(c) *0.1 N ammonium or potassium thiocyanate solution.*—Adjust by titrating against the 0.1 *N* silver nitrate solution.

(d) *Nitric acid.*—Free from the lower oxides by diluting strong nitric acid with water (4+1) and boiling until colorless.

(e) *Ferric indicator.*—A saturated solution of ferric ammonium alum.

DETERMINATION.

Weigh directly 0.2–0.5 gram of the sample in a ground-glass stoppered weighing bottle of 1 to 2 cc. capacity and of such shape that it can readily be inserted into a 60–75 cc. pressure bottle. (The weighing bottle used for molecular weight determinations by the Victor Meyer method is satisfactory for this purpose.) Transfer the weighing bottle with its contents to the pressure bottle containing 30 cc. of the alcoholic potassium hydroxide. To insure complete solution of the sample, if for carbon tetrachloride, add an additional 15–25 cc. of alcohol to the reagent already in the bottle. Remove the stopper from the weighing bottle while submerged in the reagent by working it out with a glass rod and wash the rod with a little alcohol. Stopper the bottle tightly, mix the contents thoroughly, and allow to stand about 1 hour with occasional shaking. Then place the bottle in a bath of water at room temperature. Invert a wire basket over the bottle and cover with a towel to prevent injury to the operator if the bottle should burst. Heat the bath gradually to boiling and maintain at this temperature for 3 hours. Cool, transfer the contents of the pressure bottle with the aid of water to a 200 cc. volumetric flask, removing and washing out the weighing bottle, fill to the mark with water at 20°C., and mix. Transfer (filtering if not clear) a suitable aliquot to a 400 cc. beaker; evaporate the alcohol; acidify with nitric acid, adding about 3 cc. in excess; and determine the chlorine either volumetrically by the Volhard method, or gravimetrically by precipitating and weighing as silver chloride. Make a blank test, using in the pressure bottle the same quantities of solvents and reagents as when the sample is present and apply whatever correction may be necessary. One cc. of 0.1 *N* silver nitrate solution is equivalent to 0.003979 gram of chloroform and to 0.003846 gram of carbon tetrachloride. One gram of silver chloride is equivalent to 0.2776 gram of chloroform and to 0.2683 gram of carbon tetrachloride.

METHYLENE BLUE.

The tentative method for the assay of methylene blue (p. 392) was made official (final action).

SILVER PROTEINATES.

(1) The tentative method for the determination of total silver in silver proteinates[1] was made official (final action).

(2) The tentative method for the detection of ionizable silver compounds and for the determination of ionizable silver[2], with the section, "Fold a square piece of sufficient size over one end of a glass tube, 1 inch x 4 inches, and secure it in place with a rubber band. This insures a container of the proper size.", deleted, and the sentence, "Over one end of a glass tube 10 cm. (4 inches) long and approximately 2.5 cm. (1 inch) in diameter, fold and secure by means of a rubber band a square piece of parchment paper in the form of a sack of sufficient size to hold the sample solution.", substituted therefor, was made official (final action).

(3) The following method for the determination of ionic silver by means of yeast was adopted as a tentative method.

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[1] *This Journal*, 1926, 9: 54.

[2] *Ibid.*, 55.

YEAST METHOD.—TENTATIVE.

REAGENTS.

(a) *Standard silver solution.*—Dissolve 100 milligrams of chemically pure silver nitrate in chloride-free water and dilute to 2 liters. One cc. contains 0.05 milligram of silver nitrate equivalent to 0.03 milligram of silver.

(b) *Yeast-sugar mixture.*—Triturate 8 grams of commercial pressed yeast with a 10 per cent solution of sucrose, transfer to a 200 cc. volumetric flask, and dilute to the mark with the sugar solution. This mixture should be freshly prepared.

PREPARATION OF SAMPLE.

Strong silver protein.—Weigh 1 gram into a 500 cc. volumetric flask, dilute to the mark with water, and shake well. One cc. contains 2 milligrams of sample.

Mild silver protein.—Weigh 2 grams into a 100 cc. volumetric flask, dilute to the mark with water, and shake well. One cc. contains 20 milligrams of sample.

DETERMINATION.

Place 10 cc. portions of the yeast-sugar mixture into each of a series of 15 test tubes 1.5 x 15 cm. in size. For the control test, add from a graduated pipet to the mixture in the first five tubes, 4, 4.5, 5, 5.5, and 6 cc. of the standard silver solution. Add 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 cc. of the prepared sample to the other 10 tubes. Dilute the contents of the control and sample tubes with water to 20 cc. and shake well. Fill small test tubes, 0.8 x 10 cm. in size, with the mixture from each of the larger tubes and at once invert into the corresponding larger tube, taking precaution to exclude bubbles of air from the smaller tubes. Warm the tubes in a water bath at 38°C. for 1 hour. Under the conditions of the test, gas collects in some of the small tubes and no gas is produced in the others. For comparison select the control tube and the sample tube that show no more than a small bubble of gas. If no gas forms in any of the sample tubes, repeat the test with smaller quantities of the prepared sample; if gas collects in all the sample tubes, repeat with a prepared sample of greater concentration. Calculate the percentage of ionic silver by means of the formula:

$\frac{A}{B} \times 100 =$ percentage of ionic silver by yeast, in which

A = Weight of silver in the selected control tube, and

B = Weight of sample in the selected sample tube.

NITROGLYCERIN.

The following methods for the determination of nitroglycerin were adopted as tentative methods.

NITROGLYCERIN—TENTATIVE.

REAGENTS.

(a) *Ethyl ether.*—U. S. P.

(b) *Ethyl alcohol.*—U. S. P., 95 per cent by volume.

(c) *Distilled water.*—Recently boiled and cooled and ammonia-free.

(d) *Devarda alloy.*

(e) *Aluminum wire.*—Heavy, about No. 16 gage.

(f) *Alcoholic potassium hydroxide.*—Dissolve 15 grams of potassium hydroxide in ethyl alcohol and dilute with the alcohol to 100 cc.

(g) *Standard acid solution.*—0.02 *N* hydrochloric acid or 0.02 *N* sulfuric acid.

(h) *0.02 N sodium hydroxide solution.*

(i) *Methyl red indicator.*

APPARATUS.

(a) *Kjeldahl distillation flask.*—800 cc. capacity.

(b) *Connecting bulb.*—Hopkins style, about 7.6 cm. (3 inches) in diameter. This style has a long inlet tube with an opening on the side of the tube.

(c) *Condenser.*—Water-cooled, length about 56 cm. (22 inches), and preferably of Pyrex glass.

(d) *Adapter tube.*—About 2.25 cm. (⅞ inch) in diameter at the top and with narrow outlet.

(e) *Scrubber-trap.*—Any efficient trap in which all the vapor is washed thoroughly with water before it leaves the distilling flask.

DETERMINATION.

Method I.

Place in a 50 cc. beaker a sufficient quantity of the sample, accurately weighed, to yield about ½ grain (0.0324 gram) of nitroglycerin. (If the sample consists of tablets, count those taken; if of powdered material, mix thoroughly before weighing the portion taken for analysis.) Add 10 cc. of ether and to facilitate extraction reduce the tablets to a fine powder by means of a glass stirring rod having a flattened end. After stirring thoroughly, decant the ether through a dry 7 cm. quantitative filter paper into a 250 cc. beaker containing 10 cc. of alcohol. Hold the filter paper in place in the funnel with the stirring rod and pour the ether down the rod. Make four additional extractions in the same way. Dissolve the ether-insoluble residue in a small quantity of water, transfer the solution to a separatory funnel, and extract it twice with 10 cc. portions of ether. Filter these extracts, add them to the first extracts, and to remove most of the ether evaporate the combined solutions to a volume of about 10 cc. by means of an air current from an electric fan.

Transfer the alcoholic solution containing the nitroglycerin to an 800 cc. Kjeldahl flask, rinsing the beaker first with 10 cc. of alcohol and then with a little water. Dilute to about 300 cc. with the ammonia-free water and place the flask on a wire gauze with an asbestos center. Add 2 grams of Devarda alloy (by means of a funnel), about 4 cm. of the aluminum wire, and 10–15 cc. of the alcoholic potassium hydroxide solution. Immediately after adding the alkali, place a little water in the scrubber trap and insert into the flask the rubber stopper carrying the connecting bulb and trap. Connect the outlet tube of the connecting bulb with the water-cooled condenser, which has been fixed in an upright position, fitted with the adapter dipping to the bottom of a 500 cc. Erlenmeyer flask containing a measured volume (about 25 cc.) of 0.02 *N* acid and 10–15 cc. of water, and inclined in such a way that the tip of the adapter is submerged as far as practicable under the surface of the liquid in the flask. Heat the distillation flask for about 1 hour, using a small flame and regulating the heat applied so that rapid evolution of hydrogen—but no appreciable distillation—takes place. Then gradually increase the heat until distillation begins; when active foaming ceases, continue the distillation with a large flame until all but about 40 cc. of the liquid in the distilling flask has distilled over. Lower the flame toward the end of the distillation to avoid cracking the flask. Remove the receiver containing the distillate, add sufficient methyl red indicator to make the solution red, and titrate the excess of acid with 0.02 *N* sodium hydroxide solution. From the difference between this excess and the quantity added, after making such correction as may be shown to be necessary by a blank test with the same quantity of reagents and distilled in the same manner, calculate the percentage of nitroglycerin in the sample. Each cc. of 0.02 *N* acid neutralized by the ammonia is equivalent to 0.001514 gram of nitroglycerin.

Method II.

Place in a glass-stoppered Erlenmeyer flask a sufficient quantity of the sample, accurately weighed, to yield about 1 grain (0.0648 gram) of nitroglycerin. (If the sample consists of tablets, count those taken; if of powdered material, mix thoroughly before weighing the portion taken for analysis.) Add 50 cc. of U. S. P. 95 per cent alcohol by means of a pipet. To facilitate extraction reduce the tablets to a fine powder with a glass stirring rod flattened at one end. Stopper the flask and shake Allow the mixture to settle, transfer a 25 cc. aliquot of the clear solution to an 800 cc. Kjeldahl distilling flask, dilute to about 300 cc. with the ammonia-free water, and proceed as directed in Method I.

APOMORPHINE.

The following was adopted as a tentative method for the determination of apomorphine in tablets:

APOMORPHINE.—TENTATIVE.

Weigh a number of tablets equivalent to about 0.065 gram (1 grain) of the alkaloid or of its salt and dissolve in 10 cc. of water in a separatory funnel. Add 1 cc. of a freshly prepared saturated solution of sodium bicarbonate and 25 cc. of ether, and shake the mixture. After separation, draw off the lower layer into a second separatory funnel and transfer the ethereal layer to a third funnel. Extract the mixture in the second funnel repeatedly with 15 cc. portions of ether until the alkaloid has been completely removed, using the second and first funnels alternately for the shaking, and collecting all the ethereal solution in the third. Discard the aqueous solution. Wash the ethereal solution of the alkaloid three times with 5 cc. portions of water, uniting the aqueous washings in a clean separatory funnel. Extract these washings with a little fresh ether. Discard the aqueous portion, wash the ether with water, discard the washings, and add the washed ether to the main portion of the ethereal solution. Add 20 cc. of 0.02 *N* sulfuric acid to the ethereal solution of the alkaloid in the separatory funnel and shake the mixture thoroughly. Transfer the mixture to a beaker; wash the funnel twice with 5 cc. portions of water, adding the washings to the acid liquid in the beaker; and without delay evaporate the ether at a low temperature, preferably on the water bath with the aid of a blast of air. Titrate the excess of acid with 0.02 *N* sodium hydroxide, using one drop of methyl red test solution as indicator.

One cc. of 0.02 *N* sulfuric acid is equivalent to 0.00625 gram of apomorphine hydrochloride, $C_{17}H_{17}O_2N.HCl + \frac{1}{2}H_2O$.

BARBITAL AND PHENOBARBITAL.

The tentative method for the determination of barbital and phenobarbital[1] was made official (final action).

XXXII. REFERENCE TABLES.

No additions, deletions, or other changes.

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[1] *This Journal*, 1926, 9: 51.

EGGS AND EGG PRODUCTS.

(1) The following method for the determination of the acidity of the fat was adopted as a tentative method for inclusion in the proposed chapter on Eggs and Egg Products[1] under the sub-title, "Methods for the Detection of Decomposition".

METHODS FOR THE DETECTION OF DECOMPOSITION.

ACIDITY OF FAT.—TENTATIVE.

(Not applicable to egg white.)

REAGENTS

(a) *Anhydrous ether.*—Prepared in the usual way from ordinary ethyl ether.

(b) *Benzene.*—Use the best available quality of benzene. If it is not neutral, titrate 50 cc. with the 0.05 *N* sodium ethylate and correct subsequent results accordingly.

(c) *0.05 N sodium ethylate.*—Dissolve a piece of metallic sodium, approximately 1 cc. in volume, in 800 cc. of absolute alcohol. Titrate 10 cc. of 0.1 *N* hydrochloric acid with this solution and add the calculated volume of absolute alcohol to make the solution 0.05 normal. Ascertain the normality factor by titration against 0.1 *N* hydrochloric acid on the day the solution is used.

DETERMINATION.

(a) *Dried eggs.*—Weigh in a weighed aluminum dish about 63 mm. (2½ inches) in diameter 2 grams of the powdered sample and dry at 55°C. under a pressure not exceeding 125 mm. (5 inches) of mercury. Weigh to the third decimal place at the end of 2 hours and make further weighings at ½ hour intervals until no further loss in weight occurs. Extract the dried residue with anhydrous ether, preferably in a Knorr apparatus. Carefully transfer the egg powder to a 12.5 cm. hardened filter paper, fold the paper once, place it on a 15 cm. qualitative filter paper, and roll the papers and contents into a cylinder that will fit snugly into the extraction tube, folding in one end of the cylinder to prevent loss of material. (An asbestos plug is not needed in the extraction tube, and if the extractor is working rapidly, 3 hours is sufficient to insure proper extraction.) Evaporate the ether from the extraction flask, dry the extract for 1 hour at 55°C. under a pressure not exceeding 125 mm., and weigh to the third decimal place. Dissolve the extract in 50 cc. of benzene, add 3 to 4 drops of phenolphthalein indicator, and titrate with the standard sodium ethylate solution. The end point is reached when the yellow color changes to orange. Express the result as the number of cubic centimeters of 0.05 *N* sodium ethylate required per gram of ether extract.

(*b*) *Liquid eggs.*—Weigh to the third decimal place in a weighed lead dish about 5 grams of the sample and dry as directed under (*a*). Weigh after drying for about 5 hours and thereafter, at 1 hour intervals, until no further loss in weight occurs. To prepare the dried residue for extraction with ether, place the lead dish upon a 12.5 cm. hardened filter paper, cut the sides of the dish through at four equidistant points, and flatten down. Place another similar filter paper on top of the lead dish and its contents and roll the papers and dish into a cylinder that will fit snugly into the extractor, folding in one end of the cylinder to prevent any of the egg residue from dropping into the extraction flask. Proceed thereafter as directed under dried eggs.

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[1] *This Journal*, 1926, **9**: 56.

REPORT OF THE BOARD OF EDITORS.

By R. W. **Balcom** (Bureau of Chemistry, Washington, D. C.), *Chairman.*

The members of the Board of Editors of *The Journal* were greatly saddened in April of this year by the sudden and entirely unexpected death of Mr. R. E. Doolittle who, among his many other activities connected with the work of the association, had served continuously as a member of the board since its creation was authorized by vote of the association at the meeting in 1915. The esteem in which Mr. Doolittle was held, not only by his fellow members of the Board of Editors but also by other members of the association, is well expressed by the following excerpts from letters received by the chairman from other members of the board upon their being advised of Mr. Doolittle's death. W. F. Hand wrote:

> We can but stand dismayed before the ways of fate when a man so essential to our common endeavors is taken away. Even after his position in your organization is supplied, his place will not have been filled. Vigorous qualities of mind and tender qualities of heart were centered in Doolittle. Years are required to develop a man like him. His work and wonderful influence will continue to live among us all.

H. D. Haskins said:

> To us of the Amherst institution who have known Mr. Doolittle so favorably for so many years, your letter with reference to his passing came as a distinct shock. I know of no one who has shown a deeper interest or more real affection or who has labored more earnestly for the association than has Mr. R. E. Doolittle. With reference to the personal element, his pleasing personality, his unfailing courtesy and kindly smile have always remained one of the pleasing memories of my association with the Association of Official Agricultural Chemists. He will be greatly missed by everyone.

With this brief tribute to a departed member, the matters usually covered in this report will be presented.

The year has brought no net increase in the number of subscriptions to *The Journal*, although there is evidence that it is becoming more widely known and recognized. During the year a special effort was made to bring the association's publications to the attention of those who might be interested, both in South America and Mexico. Exchanges have been effected with the Journal of the Scientific Society of Argentina and with the Journal of the Argentine Chemical Association. A good review of *Methods of Analysis* appeared in the January-February issue of the last-named publication, and the library of the association in Buenos Aires has made arrangements to acquire a complete set of this *Journal* for its files. The immediate effect of this effort in Mexico has been the increase of Mexican subscriptions to *The Journal* from two to five. One of these additional subscriptions was accompanied by an order for all back volumes of *The Journal*. In this connection it may not be out of place to mention the fact that it is becoming increasingly difficult to make up complete sets of *The Journal* for the reason that available copies of Volume I are now so scarce. For several years the editorial office has been offering the full subscription price for any copies of Volume I that might be obtained in this way, and the matter is mentioned at this time with the hope that those to whose attention this report may come will not fail to advise the board of any opportunity to obtain such copies. Still another of the additional subscriptions from Mexico came from an inquiry from a member of the teaching staff of the National College of Agriculture at Chapingo who was considering the possibility of adopting *Methods of Analysis* as a textbook for his classes. The price was more than he thought his students could afford to pay, but the inquiry resulted in the purchase of two copies of *Methods of Analysis* and a subscription to *The Journal.*

The total circulation of *The Journal* at the present time is 860 copies, of which 830 are paid subscriptions. Our limited exchanges require 14 copies; complimentary copies number 12; and 4 copies are sent to those who are using *The Journal* for advertising purposes. The net decrease of eight subscriptions in the United States during the year has been offset by the same net increase in subscriptions from other countries so that the number of paid subscriptions is the same this year as it was a year ago. It is believed, however, that the subscription list is becoming more and more permanent—that is, that the number of subscriptions that have to be taken from the mailing list each year, for one reason or another, is growing less, and it is hoped that the time will soon come when new subscriptions entered will more than counterbalance cancellations. The total circulation of *The Journal* is now as high as it has ever been, but the board is not content with this and will not be satisfied until it can report a steady gain in circulation each year, even though such gain cannot be expected to be large.

The sale of *Methods of Analysis* during the year has met all expectations. More than a thousand copies have been sold since the date of the last meeting. Total sales, since the 1925 edition was ready for distribution, are now close to 2400 copies. There remain of the 3000 copies first printed only about 600 copies, the last 475 of which are now in process of being bound. The bill for binding these 475 copies will be something like $300. With the exception of this bill, all expenses in connection with the preparation of the first 3000 copies of the 1925 edition of *Methods of Analysis*, including composition, press work, stock, binding, etc., have been paid. It is almost certain that a second printing will be necessary before the expiration of another year. It is from the sale of copies from this second printing that the association may hope to accumulate a reserve fund for assistance in the financing of a later edition of *Methods of Analysis*, or for other purposes, as the cost per copy of the second printing, which will include only stock, press work, and binding, should not be more than about half of the cost per copy of the first 3000 copies.

The detailed financial statement of receipts and disbursements from October 15, 1925 to October 1, 1926, is appended.

FINANCIAL REPORT OF THE SECRETARY-TREASURER FROM OCTOBER 15, 1925, TO OCTOBER 1, 1926.

By W. W. SKINNER (Bureau of Chemistry, Washington, D. C.).

RECEIPTS.

1925			
Oct. 15	Bank balance	$898.96	
	1925 dues received too late for inclusion in 1925 report, 1 at $5.00	5.00	
	1926 dues from institutional members, 61 at $5.00	305.00	
1926			
Aug. 9	Reimbursement from *Journal* account for loan of July 24	19.30	
	Plus check redeposited	5 00	
	Total		$1,233.26

DISBURSEMENTS.

		Amount	Check No.	
1925				
Oct. 31	Marian E. Lapp, 1925 meeting expenses	$25 00	46	
Nov. 10	Bastian Bros. Co., bill of 10-16-25	11.37	47	
1926				
Feb. 1	Industrial Printing Co., stationery bill of 12-31-25	70.88	48	
Apr. 27	Geo. C. Shaffer, flowers for Mr. Doolittle's funeral	20.00	49	
July 24	Postmaster, Washington, D. C., loan to *Journal* account in absence of Dr. Balcom	19.30	50	
Sept. 29	Marian E. Lapp, mailing programs, 1926 meeting	25.00	51	
	Plus check returned for endorsement	5.00		
	Total	$176.55		
Oct. 1	Bank balance	1,056.71		
	Total			$1,233.26

FINANCIAL REPORT ON PUBLICATIONS FROM

By R. W. Balcom (Bureau of Chemistry,

RECEIPTS.

Methods of Analysis.

Number	Price each	Total	
49	$5.50	$269.50	
743	5.00	3,715.00	
116	4.40	510.40	
326	4.00	1,304.00	
		$5,798.90	
Plus gain on exchange		.21	
Total			$5,799.11

Journal Subscriptions.

Number	Price each	Total	
31	$8.25	$255.75	
69	7.50	517.50	
9	6.60	59.40	
42	6.00	252.00	
15	5.50	82.50	
80	5.00	400.00	
52	4.40	228.80	
124	4.00	496.00	
		$2,291.95	
Plus payment on one subscription from bankruptcy sale, Jersey Cereal Food Co., Pittsburgh, Pa.		.79	
Total			2,292.74

Advertisements.

Number	Price each	Total	
5	$15.00	$75.00	
15	25.00	375.00	
Total			450.00

Reprints.

Mitchell and Alfend	$6.00	
C. A. Browne	6.00	
P. B. Clark	3.21	
D. B. Dill	3.00	
Mitchell and Alfend	4.00	
Total		22.21

Miscellaneous.

1 A. O. A. C. Journal	$1.50	
1 Book on Chemistry of Wheat Flour	3.50	
1 Milling Chemistry Book	1.00	
Total		6.00

Total for Methods, Journal, Ads, Reprints, and Miscellaneous	$8,570.06
Minus re-deposited checks	57.40
	$8,512.66
Plus Bank Balance of October 15, 1925	571.61
Total	$9,084.27

OCTOBER 15, 1925, TO OCTOBER 1, 1926.

Washington, D. C.), *Chairman, Board of Editors.*

DISBURSEMENTS.

1925		Amount	Check No.
Oct. 15	Estelle L. Milne, office expenses	$50.00	149
Nov. 10	Industrial Printing Co., on account, bill of 7-13-25	1,000 00	150
Nov. 13	Estelle L. Milne, office expenses	50 00	151
Nov. 30	Dorothy M. Cosford, back number of *Journal*	1 25	152
Dec. 8	Industrial Printing Co., on account, bill of 7-13-25	1,000 00	153
Dec. 17	Citro Chemical Co., refund for overpayment on *Methods*	.50	154
Dec. 22	Estelle L. Milne, office expenses	50.00	155
Dec. 22	Postmaster, Washington, D. C., box rent, quarter ending 3-31-26	2.00	156
1926			
Jan. 19	Industrial Printing Co., bill of 11-12-25	1,266.68	157
Feb. 8	Estelle L. Milne, office expenses	50.00	158
Feb. 12	G. W. Hoover, back numbers of *Journal*	8.00	159
Feb. 15	B. G. Hartmann, back numbers of *Journal*	6 50	160
Feb. 17	Industrial Printing Co., on account, bill of 7-13-25	1,000.00	161
Feb. 18	Industrial Printing Co., bill of 1-30-25	48 88	162
Mar. 20	Williams & Wilkins, back numbers of *Journal*	15.00	163
Mar. 26	Estelle L. Milne, office expenses	50 00	164
Mar. 27	Industrial Printing Co., on account, bill of 7-13-25.	1,000 00	165
Apr 1	Postmaster, Washington, D. C., box rent, quarter ending 6-30-26	2.00	166
Apr. 15	D. Van Nostrand Co., refund on foreign subscription	1.20	167
Apr. 17	R. H. Carr, back numbers of *Journal*	7.87	168
Apr. 30	Industrial Printing Co., bills of 3-18-26 and 4-12-26	68 88	169
May 17	Estelle L. Milne, office expenses	50.00	170
May 25	Richmond Br., Tobacco By-Products & Chemical Corp., refund on *Methods*	5.00	171
May 26	University of Rochester, Library, back number of *Journal*	1.25	172
May 27	United Drug Co., back numbers of *Journal*	8.13	173
June 3	Industrial Printing Co., final payment on bill of 7-13-25 on *Methods*	1,176.00	174
June 7	Chemical Catalog Co., "Book on Chemistry of Wheat Flour"	3 50	175
June 7	National Miller, "Milling Chemistry Book"	1.00	176
June 8	C. A. Browne, picture of R. E. Doolittle	2.00	177
June 18	Cash, office expenses	50.00	178
June 22	Postmaster, Washington, D. C., box rent, quarter ending 9-30-26	2.00	179
July 28	W. W. Skinner, reimbursement on payment for 5,000 window envelopes	19.30	180
July 28	Industrial Printing Co., bill of 3-9-26	1,017.50	181
Aug. 2	Cash, office expenses	50.00	182
Aug. 10	Government Printing Office, Postal Guide Book	1.00	183
Aug. 18	Postmaster, Pa. Ave. Station, balance payment on 5,000 window envelopes	100.00	184
Aug. 23	Bank of Montreal, account of Ernest Childs, refund on *Methods* returned	5.00	185
Sept. 8	Williams & Wilkins, back numbers of *Journal*	15 00	186
Sept. 15	Ware Bros. Co., refund for duplicate payment on *Methods*	4.00	187
Sept. 21	Industrial Printing Co., bills of 6-5-26 and 6-14-26	346.95	188
Sept. 21	Postmaster, Washington, D. C., box rent, quarter ending 12-31-26	2.00	189
	Plus bank balance, October 1, 1926	545.88	
		$9,084.27	

No report was made by the Committee on Quartz Plate Standardization and Normal Weight.

F. W. Zerban. Mr. Chairman, I wish to state that Dr. Browne and other members of the committee have started to investigate this work, and it is hoped that we may submit a report at the next meeting of the association.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS[1].

The committee recommends the following definitions and interpretation of terms:

For Final Adoption as Official.

1. FERTILIZER FORMULA.

The term *fertilizer formula* shall be interpreted as expressing the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16 per cent acid phosphate, 800 pounds of 9–20 tankage, and 400 pounds of sulfate of potash-magnesia.

2. ANALYSIS.

The word *analysis*, as applied to fertilizers, shall designate the percentage composition of the product expressed in terms of nitrogen or ammonia, phosphoric acid, and potash in their various forms.

3. BRAND AND BRAND NAME.

A *brand* is a term, design, or trade mark used in conection with one or several grades of fertilizers.

A *brand name* is a specific designation applied to an individual fertilizer.

4. UNIT.

A *unit* of plant food is twenty (20) pounds, or one per cent (1%) of a ton.

5. LEACHED WOOD ASHES.

Leached wood ashes are defined as ashes resulting from burning unleached wood, but as having had part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent.

6. ASHES FROM LEACHED WOOD.

Ashes from leached wood are defined as unleached ashes resulting from burning wood that has been exposed to or digested in water or other liquid solvent, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed.

7. DISSOLVED BONE.

Dissolved bone is defined as a ground bone or bone meal that has been treated with sulfuric acid.

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[1] Presented by C. H. Jones, who presided at the meeting of the committee in the absence of H. D. Haskins.

8. FORM OF NITROGEN IN CYANAMIDE.

The nitrogen in calcium cyanamide shall be considered as being of organic nature.

Second Recommendation as Tentative.

1. MEANING OF TERM "FINELY GROUND".

The term *finely ground* in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more should pass a 100-, and ninety per cent (90%) or more should pass a 50-mesh sieve.

2. NITRATE OF POTASH.

Nitrate of potash is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash (K_2O).

3. INTERPRETATION OF BRAND NAME TO INCLUDE THE ANALYSIS OR GRADE OF FERTILIZER.

The committee recommends and urges the practice of including the analysis or grade of fertilizer with the brand name, both by the manufacturer on sacks and in printed literature and by the control official in his reports and publications.

4. ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS.

The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen, and do not show the percentage availability of the material. The available nitrogen of any product can be measured only after carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. In the event of a total nitrogen exceeding the minimum guarantee, accompanied by a low activity of the insoluble nitrogen, the over-run *may* be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods before classifying as inferior.

Amended Tentative Interpretations and Definitions.

1. MAXIMUM AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE.

The *chlorine* in mixed fertilizers in which the potash is claimed as sulfate shall not exceed five-tenths of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition for sulfate of potash as formulated by the committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5.

2. DEFINITION OF PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALINE SALTS CONTAINING POTASH.

These products are *not* potassium phosphate. They may be called non-acid phosphates with potash.

3. MURIATE OF POTASH.

Muriate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) largely as chloride.

4. SULFATE OF POTASH.

Sulfate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) largely as sulfate, and not more than two and one-half per cent (2.5%) of chlorine.

5. UNLEACHED WOOD ASHES.

Unleached wood ashes are defined as ashes resulting from burning unleached wood and that have not had any part of their plant food extracted by contact with water or other solvent, and shall contain four per cent (4%) or more of water-soluble potash (K_2O).

First Recommendation as Tentative.

1. NITRATE OF SODA.

Nitrate of soda is a nitrogen salt containing not less than fifteen per cent (15%) of nitrogen largely as sodium nitrate.

2. KAINIT.

Kainit is a natural potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia, with not less than twelve per cent (12%) of potash (K_2O).

3. FERTILIZER GRADE.

The *grade of a fertilizer* shall represent the minimum guarantee of its plant food expressed in terms of ammonia (or nitrogen when the custom prevails of using this word), available phosphoric acid, and water-soluble potash.

4. DRIED BLOOD.

Dried blood is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic form.

5. GROUND STEAMED BONE.

Ground steamed bone is a product resulting from grinding animal bones that have been previously steamed under pressure.

6. GROUND RAW BONE.

Ground raw bone is a product secured by drying and grinding animal bones not previously steamed under pressure.

The following topics have been proposed for future consideration:

1. Tankage.—The term *tankage* (without qualification) is restricted to slaughter house tankage. If the term *tankage* is used as a part of the name designating other processed material, it shall be accompanied by a qualifying word or words, which shall not be misleading.
2. Uniformity of Fertilizer Bulletins.
3. Significance of the words "blood" and "bone" as a part of the brand name of a mixed fertilizer.—The words *blood* and *bone* shall not be used as a part of the brand name of a mixed fertilizer unless all organic nitrogen present in the mixture is derived from blood and bone.
4. Definition of the following lime products as applied to control service work: agricultural lime, hydrated lime, carbonate of lime, sulfate of lime (land plaster).
5. Uniform order and terms in expressing the grade of a fertilizer.

H. D. HASKINS, J. W. KELLOGG,
R. N. BRACKETT, C. H. JONES.
G. S. FRAPS,
Committee on Definition of Terms and Interpretation of Results on Fertilizers.

Approved.

REPORT OF COMMITTEE ON REVISION OF METHODS OF SOIL ANALYSIS.

The personnel of the Committee on Revision of Methods of Soil Analysis is so geographically distributed that its work is done to a great extent by correspondence. This year the present method for the determination of manganese, which was found to be in error, was considered. The second proposition that came up related to the desirability of the determination of the so-called less abundant elements, such as copper and zinc in soils. It is interesting to note that the occurrence of such elements in plant ash has also been brought to the attention of Committee A, through recommendations from the appropriate referee.

Another topic considered was the desirability of some research leading to the question of potash availability by some six methods to measure the so-called interchangeable or replaceable potash in soils that have been treated for a period of years. A considerable amount of work in this connection was done by two men in the chairman's laboratory this year. With the concurrence of the committee, action was taken by the referee and embodied in his report. That report has been presented to this committee and placed before Committee A as representing the recommendations of the Committee on Revision of Methods of Soil Analysis.

W. H. MacIntire, J. A. Bizzell,
A. W. Blair, Robert Stewart.
A. G. McCall,
Committee on Revision of Methods of Soil Analysis.

Approved.

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES.

The report of the Committee on Recommendations of Referees is very completely set forth in the reports of Sub-Committees A, B, and C, and little can be added beyond an expression of appreciation of the loyal co-operation of referees and associate referees. The large amount of work done, and the general excellence of it, are both highly commendable.

It seems necessary, however, to urge again that reports be submitted as nearly as possible in accord with the directions contained in the call for the annual meeting as sent out by the secretary. The sub-committees endeavor to give each report submitted careful consideration, and to this end it is evidently important that reports should be in their hands in time for such consideration. This point is urged quite as much in fairness to referees and associate referees themselves as to the committee, because late reports may not receive the attention they deserve. It has been suggested that the work of the committee will be greatly facilitated if, in addition to the duplicate copies of reports required, two additional copies of the recommendations contained therein are submitted.

The committee wishes to suggest that in view of the increasing use and applicability of microchemical methods of analysis, referees and associate referees consider such methods in the studies for which they are appointed.

E. M. Bailey, *Acting Chairman.*

Approved.

REPORT OF SUB-COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By B. B. Ross (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman.*

[To this committee are referred reports on the following subjects: Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides; soils and liming materials (reaction value of soils, liming materials); feeding stuffs (stock feed adulteration, mineral mixed feeds, determination of moisture); sugars and sugar products (maple products; starch conversion products; drying, densimetric, and refractometric methods for determination of solids; polariscopic methods; chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, potash); plants.]

WATERS, BRINE, AND SALT.

It is recommended—

(1) That the following changes be made in the method for the determination of manganese by the bismuthate method as given on p. 101 of *Methods of Analysis:* In lines 8 and 17 of section 75, change the word "bisulfate" to "bisulfite".

Approved.

(2) That the referee study methods for the analysis of salt, giving particular attention to methods for the determination of ingredients which are added to prevent caking and for the determination of added iodides.

Approved.

TANNING MATERIALS AND LEATHER.

It is recommended—

(1) That the tentative method[1] for the determination of moisture be dropped.

Approved.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 79.

(2) That the toluene distillation method (see p. 31) for the determination of moisture be adopted as tentative.

Approved.

INSECTICIDES AND FUNGICIDES.

It is recommended—

(1) That the official methods for the determinations of cyanogen and chlorine in sodium and potassium cyanides[1] be dropped (first action).

Approved.

(2) That Method II for the determination of cyanogen in sodium and potassium cyanides (see p. 27) be adopted as official (first action).

Approved.

(3) That Methods I and II for the determination of chlorine in sodium and potassium cyanides (see p. 28) be adopted as official (first action).

Approved.

(4) That Method I for the determination of cyanogen in calcium cyanide (see p. 29) be adopted as official (first action).

Approved.

(5) That Methods I and II for the determination of chlorine in calcium cyanide (see p. 29) be adopted as official (first action).

Approved.

(6) That the official method for the determination of moisture in soap[1] be dropped (first action).

Approved.

(7) That the xylene distillation method for the determination of water in soap[2] be adopted as official (first action).

Approved.

(8) That the methods for the determination of water, total oil, and ash in mineral oil-soap emulsions[3] be adopted as official (first action).

Approved.

(9) That no further study be made of Method I for the determination of soap in mineral oil-soap emulsions[4] and that this tentative method be dropped.

Approved.

(10) That Method II for the determination of soap in mineral oil-soap emulsions[5] be adopted as official, with the following note appended: "Error will result in this method if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid" (first action).

Approved.

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[1] *Methods of Analysis, A. O A. C.*, 1925, 65.
[2] *This Journal*, 1926, 9: 27.
[3] *Ibid.*, 28.
[4] *Ibid.*, 129.
[5] *Ibid*, 28, 129.

(11) That in Method I for the determination of unsulfonated residue in mineral oils, as given in the report of the referee (see p. 30), the following be substituted for the first sentence and that the method then be adopted as official for the determination of unsulfonated residue in mineral oils and the recovered oil obtained in the analysis of mineral oil-soap emulsions (final action).

With a pipet measure 5 cc. of the oil into a Babcock cream bottle. (After preliminary draining in the case of heavy oils, to reduce the viscosity, warm the pipet by drawing it several times through the flame of a Bunsen burner and then drain thoroughly.) In lieu of this procedure determine the density of the oil and weigh the equivalent of 5 cc.

Approved.

(Recommendations 7, 8, 9, 10, and 11 cover, with some revision, methods that were adopted as tentative at the 1925 meeting.)

SOILS AND LIMING MATERIALS.

It is recommended—

(1) That an associate referee be appointed to study the present procedure in the determination of manganese, and to formulate, if possible, a more accurate procedure.

Approved.

(2) That in view of the more recent work upon the occurrence of certain less common elements, such as arsenic, copper, tin, and zinc, in some soils, the associate referee appointed to study manganese be requested to include such studies as a part of his efforts and that he be designated as Associate Referee on Manganese and the Less Abundant Elements, or by a similar title.

Approved.

(3) That the work of investigation of the solubility of soil potassium be continued with a view to a further report by the referee.

Approved.

REACTION VALUE OF SOILS.

It is recommended that the recommendations of the associate referees be approved with the following provisos:

(a) That the recommendations of the Associate Referee on Reaction Value of Soils be formulated into definite directions by the Committee on Revision of Soil Analysis.

(b) That determined reaction values be expressed in terms of "specific acidity" and "specific alkalinity," and that corresponding exponential values be given in brackets and a table of corresponding values for the two methods be appended.

Approved.

LIMING MATERIALS

It is recommended that recognition be given to the advisability of insuring against the vitiating factor of sulfides in calcined and hydrated limes, pyrite in limestone, and sphalerite in dolomites, in the determination of carbon dioxide and that the Committee on Revision of Methods of Soil Analysis be requested to insert such provision in the present method.

Approved.

STOCK FEED ADULTERATION.

It is recommended—

(1) That the method for the determination of oat hulls in oat feeds[1] (see p. 32) be adopted as tentative.

Approved.

(2) That the study of microanalytical methods as related to feeding stuffs be continued.

Approved.

MINERAL MIXED FEEDS.

It is recommended—

(1) That the method proposed by the associate referee for iodine in mineral feeds be studied during the coming year and that samples be submitted to collaborators for analysis.

Approved.

(2) That the method proposed by the associate referee for lime (CaO) be further studied and that samples be submitted to collaborators for analysis.

Approved.

DETERMINATION OF MOISTURE.

It is recommended that the Bidwell-Sterling distillation method for the determination of moisture[2] be adopted as official (first action).

Approved.

SUGARS AND SUGAR PRODUCTS.

It is recommended that the program of work in the several subdivisions be continued along the lines already suggested and approved at last year's meeting[3].

Approved.

MAPLE PRODUCTS.

No report was submitted.

STARCH CONVERSION PRODUCTS.

No report was submitted.

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[1] *This Journal*, 1926, 9: 149.
[2] *Ibid.*, 30.
[3] *Ibid.*, 72.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS FOR DETERMINATION OF SOLIDS.

It is recommended—

(1) That the work for next year consist of (1) obtaining further densimetric and refractometric data on pure dextrose, fructose, and invert sugar solutions covering the total range of their solubility, and (2) that the application of such information be made to sugar mixtures of known composition and finally to sugar-house products.

Approved.

(2) That when opportunity permits, similar information be obtained on the various other sugars that may be encountered by the Federal or public analyst. This list would include maltose, lactose, and perhaps raffinose.

Approved.

POLARISCOPIC METHODS.

It is recommended that the work outlined in Recommendations (4) and (5)[1] of the associate referee's report for 1925 and not finished during the present year be completed during the coming year.

Approved.

CHEMICAL METHODS FOR REDUCING SUGARS.

No report was submitted.

FERTILIZERS.

PHOSPHORIC ACID.

It is recommended—

(1) That the calcium chloride method for preparing ammonium citrate[2] be eliminated from the methods (first action).

Approved.

(2) That the study of the effect of varying the conditions under which precipitation is made with magnesia mixture be continued for another year.

Approved.

(3) That the recommendations of the collaborators, as given in the report of the associate referee, be followed in the preparation of the standards used, and that the standard, or standards, so prepared be tested at intervals over a sufficient period to insure the constancy of their composition.

Approved.

(4) That the words "dilute to 1 liter" in the second of the alternative methods[3] for the preparation of magnesia mixture be changed to read "proceed as in (1)".

Approved.

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[1] *This Journal*, 1926, 9: 73.

[2] *Methods of Analysis, A. O. A. C.*, 1925, 4, Section 13 (2).

[3] *Ibid.*, 2, Section 5 (c), last line.

NITROGEN.

It is recommended—

(1) That the absolute or cupric oxide method for nitrogen[1] be removed from the methods for fertilizers (first action).

Approved.

(2) That an associate referee be appointed to study the permanganate methods for nitrogen in fertilizers[2], and that such modifications as appear desirable be recommended.

Approved.

(3) That further work on the Breckenridge method be discontinued for the present.

Approved.

(4) That the Jones method for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide and urea be further studied.

Approved.

POTASH.

It is recommended—

(1) That a study be made of the use of calcium carbonate in preparing the solution for the determination of potash.

Approved.

(2) That work on a method for chlorine in fertilizers be continued.

Approved.

PLANTS.

It is recommended—

(1) That the method for the determination of iron and aluminum be further studied.

Approved.

(2) That an associate referee be appointed to study methods for the determination of copper, zinc, nickel, cobalt, and other so-called "rare elements".

Approved.

(3) That an associate referee be appointed to study methods for the determination of total chlorine.

Approved.

(4) That an associate referee be appointed to study methods for the preparation of samples for analysis.

Approved.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 9.

[2] *Ibid.*, 12.

REPORT OF SUB-COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By H. C. LYTHGOE (Massachusetts Department of Public Health, Boston, Mass.), *Chairman.*

[To this committee are referred reports on the following subjects: Testing chemical reagents, spices and other condiments, naval stores (turpentine), specific gravity and alcohol, drugs (acetylsalicylic acid, alcohol in drugs, arsenicals, cocaine, chaulmoogra oil, crude drugs, chloroform and carbon tetrachloride, ipecac alkaloids, radio activity in drugs and water, laxatives and bitter tonics, mercurials, pyramidon, microchemical alkaloid methods, silver proteinates, nitroglycerin, terpin hydrate, apomorphine, santonin, ether, bioassay of drugs).]

TESTING CHEMICAL REAGENTS.

It is recommended that this subject be dropped, but that a report upon testing chemical reagents be submitted by the Bureau of Chemistry as a contributed paper.

Approved.

SPICES AND OTHER CONDIMENTS.

No report was submitted. The committee recommends a repetition of the recommendation of last year relative to salad dressing[1].

Approved.

NAVAL STORES.

No report was presented. The committee recommends further study as outlined last year[2].

Approved.

SPECIFIC GRAVITY AND ALCOHOL.

No report was submitted.

The committee recommends—

(1) That further study be made of the correlation of refractometric and pycnometric methods for the determination of alcohol.

Approved.

(2) That the referee consider the practicability and desirability of recalculating the alcohol tables to involve any or all of the following features: (1) Specific gravity figures arranged in accordance with a definite interval in gravity; (2) per cent by volume to be based upon volume at 15.56/15.56°C. (60/60°F.); (3) substitution of apparent for true specific gravity; (4) tables to be arranged for various working temperatures as in the case of the refractometric tables.

Approved.

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[1] *This Journal*, 1925, **8**: 264.

[2] *Ibid.*, 1926, **9**: 75.

DRUGS.

ALCOHOL IN DRUGS.

It is recommended that the referee study methods for the examination of peculiar mixtures of alcohol with other substances, such as ether, acetone, ethyl acetate, normal propyl alcohol, isopropyl alcohol, methyl alcohol, benzol, etc.

Approved.

ACETYLSALICYLIC ACID.

It is recommended—

(1) That the tentative method (Method II, revised)[1] for the determination of combined acetic acid be made official (final action).

Approved.

(2) That the bromine method for total salicylates[2] be made official (final action).

Approved.

(3) That the tentative double titration method for the determination of acetylsalicylic acid[3] be modified to include the wet extraction method for preparation of sample and the single titration procedure for the final determination as described by the referee in his report last year and that the modified method be made official (final action).

Approved.

(4) That the tentative qualitative test for free salicylic acid in acetylsalicylic acid[4] be made official (final action).

Approved.

ARSENICALS.

It is recommended—

(1) That the method for arsenic in sodium cacodylate[5], as proposed last year, be studied with a view to making it official.

Approved.

(2) That the method for arsenic in iron-arsenic tablets (see p. 44) be adopted as tentative.

Approved.

CAMPHOR AND MONOBROMATED CAMPHOR.

It is recommended that Method I[6], now tentative, for the determination of monobromated camphor in tablets be made official (final action).

Approved.

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[1] *This Journal*, 1925, 8, 506: 1926, 9: 49.
[2] *Methods of Analysis, A. O A. C.*, 1925, 388.
[3] *This Journal*, 1926, 9: 49, 279.
[4] *Methods of Analysis, A. O. A. C.*, 1925, 387.
[5] *This Journal*, 1926, 9: 51, 287.
[6] *Methods of Analysis, A. O. A. C.*, 1925, 393.

COCAINE.

It is recommended that a study be made of the various methods reported and recommended by the referee.

Approved.

CHAULMOOGRA OIL.

It is recommended that this subject be given further study as outlined by the associate referee last year[1].

CRUDE DRUGS.

No report was submitted.

CHLOROFORM AND CARBON TETRACHLORIDE.

It is recommended—

(1) That the methods for the determination of chloroform and carbon tetrachloride submitted by the associate referee be made tentative (see p. 45).

Approved.

(2) That the methods for the separation of chloroform in mixtures submitted by the associate referee be studied collaboratively.

Approved.

IPECAC ALKALOIDS.

It is recommended that further study be made of Methods 1 and 2 reported by the associate referee, and that a collaborative study be made of the purification method of Palkin and Watkins[2].

Approved.

RADIO ACTIVITY IN DRUGS AND WATER.

It is recommended—

(1) That a collaborative study be made of the method recommended by the associate referee with a view to its adoption as tentative.

Approved.

(2) That the associate referee prepare a description of the preparation of a standard stock solution of radium.

Approved.

LAXATIVES AND BITTER TONICS.

It is recommended that work be continued as outlined in 1924[3].

Approved.

MERCURIALS.

It is recommended that further study be made of the method reported by the associate referee in connection with other methods with a view to shortening the procedure.

Approved.

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[1] *This Journal*, 1926, **9**: 77, 291.

[2] *Ibid.*, 301; *Ind Eng. Chem.*, 1925, **17**: 612.

[3] *This Journal*, 1925, **8**: 267.

METHYLENE BLUE.

It is recommended that the iodometric method for the determination of methylene blue[1] be adopted as official (final action).

Approved.

PYRAMIDON.

It is recommended that further study be made of the present tentative quantitative methods[2] with a view to making them official.

Approved.

MICROCHEMICAL ALKALOID METHODS.

It is recommended that further study be given to microchemical methods for alkaloids with the view to including a systematic description and diagrams of the more important alkaloids.

Approved.

SILVER PROTEINATES.

It is recommended—

(1) That the tentative method for the determination of total silver[3] be made official (final action).

Approved.

(2) That the tentative qualitative and quantitative methods for ionizable silver compounds[4], but with the description of the dialyzing tube amended as suggested by the associate referee (see p. 46), be made official (final action).

Approved.

(3) That the method for the determination of ionizable silver by yeast[5] (see p. 47) be made tentative.

Approved.

NITROGLYCERIN.

It is recommended that Methods (a) and (c), as reported by the associate referee, be adopted as tentative (see p. 47).

Approved.

TERPIN HYDRATE.

It is recommended that work upon this subject be continued.

Approved.

APOMORPHINE.

It is recommended that Method 1, reported by the referee, be adopted as tentative (see p. 49).

Approved.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 392.
[2] *This Journal*, 1925, 8: 546.
[3] *Ibid.*, 1926, 9: 54.
[4] *Ibid.*, 55.
[5] *Ibid.*, 314.

SANTONIN.

It is recommended that collaborative study be given to methods outlined by the referee for 1925[1].

Approved.

ETHER.

It is recommended that work be continued along the line suggested by the associate referee.

Approved.

BIOASSAY OF DRUGS.

It is recommended that the work on the bioassay of drugs be continued, and that the method reported by the associate referee for midriatic alkaloids be investigated.

Approved.

BARBITAL AND PHENOBARBITAL.

It is recommended that the tentative method for the estimation of barbital and phenobarbital[2] be adopted as official (final action).

Approved.

It is recommended that work upon apomorphine and nitroglycerin be discontinued.

Approved.

REPORT OF SUB-COMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By E. M. Bailey (Agricultural Experiment Station, New Haven, Conn.), *Acting Chairman.*

[To this committee are referred reports on the following subjects: Dairy products (butter, cheese, malted milk, dried milk, ice cream), fats and oils, baking powders and baking chemicals, eggs and egg products (total solids and acidity of lipoids, detection of decomposition, water-soluble protein nitrogen precipitable by 40 per cent alcohol, unsaponifiable matter, and ash), food preservatives, coloring matters in foods, metals in foods (zinc in dried eggs), fruits and fruit products (water in grape juice, ash in fruit products, fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat products), gelatin, cacao products (microscopical methods, crude fiber, cacao butter), cereal foods (sampling of flour, moisture in flour and in alimentary pastes, ash in flour and gasoline color value, glutenin in flour, hydrogen-ion concentration of flour, gluten in flour, diastatic value of flour, starch in flour, chlorine in bleached flour, experimental baking tests, unsaponifiable matter and fat in flour and in alimentary pastes, methods for bread analysis).]

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[1] *This Journal*, 1926, 9: 326.
[2] *Ibid.*, 51.

DAIRY PRODUCTS.

It is recommended—

(1) That the cryoscopic method for the determination of added water in milk[1] be adopted as an official method for the determination of added water in cream, the percentage of added water being ascertained by the following formula:

$$W = \frac{\%\ \text{Serum in Cream}\ (T - T')}{T},\ \text{in which}$$

W = the percentage of added water;
T = the freezing point of undiluted cream (-0.550°C.);
T' = the observed freezing point of the given sample; and
$\%\ \mathit{Serum} = 100\% - (\%\ \text{fat} + \%\ \text{protein})$.
If protein is not determined it may be assumed to be 38 per cent of the solids-not-fat.

This recommendation is for first action as official.

Approved.

(2) That the methods for determining specific gravity of milk[2] be studied with a view to more detailed statements of procedure.

Approved.

(3) That an associate referee be appointed to study methods for the determination of milk proteins.

This recommendation was referred to the Executive Committee and approved.

(4) That an associate referee be appointed to study qualitative tests applicable to milk and milk products.

This recommendation was referred to the Executive Committee and was approved.

BUTTER.

It is recommended—

(1) That work on the preparation of butter samples for analysis be continued.

Approved.

(2) That the official methods for the analysis of butter be studied collaboratively in comparison with certain new methods cited by the associate referee.

Approved.

(3) The associate referee makes further recommendations relating to the sampling of butter.

The committee approves of these recommendations with the reservation that this question be referred to the Committee on Sampling, citing to that committee the data obtained on this subject by the associate referee.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 265.
[2] *Ibid.*, 259.

Recommendation of the committee approved.

(4) That it be ascertained whether or not tin containers are suitable for butter samples and may be used in place of glass containers.

The committee approves this recommendation with the reservation noted in (3).

Recommendation of the committee approved.

CHEESE.

It is recommended—

(1) That the method for the determination of moisture in cheese[1] be adopted as official (final action).

Approved.

(2) That methods for the detection of preservatives, coloring matters, emulsifying agents, or other added substances in cheese be further studied.

Approved.

MALTED MILK.

It is recommended that the study of methods for the analysis of malted milk be continued.

Approved.

DRIED MILK.

It is recommended—

(1) That the method for the preparation of the sample, as described for malted milk[2], be adopted as tentative for dried milk.

Approved.

(2) That the methods for the determination of protein and of ash, as described for malted milk[2], be adopted as tentative for dried milk.

Approved.

(3) The committee recommends further study of methods for the determinations of fat and of moisture with a view to harmonizing these methods with those described for malted milk, if feasible.

Approved.

ICE CREAM.

The committee recommends that the associate referee continue his study of methods for the analysis of ice cream and submit definite recommendations if possible.

Approved.

FATS AND OILS.

It is recommended—

(1) That the official method for the determination of unsaponifiable residue in fats and oils[3] be dropped (final action).

Approved.

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[1] *This Journal*, 1926, 9: 44.

[2] *Methods of Analysis, A. O. A. C.*, 1925, 275.

[3] *Ibid.*, 295.

(2) That the F. A. C. method for the determination of unsaponifiable matter, with a slight modification since previous publication[1] (see p. 35), be made official (final action).

Approved.

(3) That the study of the Thomas and Yu method[2] for the detection and determination of peanut oil alone or in presence of other oils be continued.

Approved.

(4) That the André-Cook method for the determination of acetylation value, as modified in technique by the referee (see p. 35), be adopted as official (first action).

Approved.

(5) The committee recommends, and the referee concurs, that the present official method for the determination of acetyl value[3] be dropped (first action).

Approved.

BAKING POWDERS AND BAKING CHEMICALS.

It is recommended—

(1) That the present tentative electrolytic method for the determination of lead[4] be adopted as official (final action).

Approved.

(2) That the present tentative gasometric method for the determination of total carbon dioxide and of residual carbon dioxide[5], modified as described by the referee this year (see p. 36), be adopted as an official method (first action).

Approved.

(3) That methods for the determinations of ortho-pyro and metaphosphates in presence of one another be further studied.

Approved.

EGGS AND EGG PRODUCTS.

It is recommended—

(1) That further study be given to the vacuum oven at 98°C., the air oven, and the vacuum oven at 55°C. methods for the determination of total solids to ascertain the efficiency of each with respect to actual moisture removal.

Approved.

(2) That studies be made by the associate referee for the purpose of perfecting methods for the determination of:

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[1] *This Journal*, 1926, 9· 45.

[2] *J. Am. Chem. Soc.*, 1923, 45· 113.

[3] *Methods of Analysis, A O A. C.*, 1925, 293.

[4] *Ibid.*, 310.

[5] *Ibid.*, 305.

(a) water-soluble protein-nitrogen precipitable by 40 per cent alcohol,
(b) ash, and
(c) unsaponifiable matter,

and to include, if possible, collaborative studies of these methods and of methods for the determination of fat (acid hydrolysis) and of lipoids and lipoid P_2O_5.

Approved.

(3) That the method for the determination of acidity of the fat, as described in the report of associate referee Macomber (see p. 50), be adopted as a tentative method; and that this method be included in *Methods of Analysis* in the chapter on Eggs and Egg Products under the sub-title "Methods for the Detection of Decomposition"; and that studies of other methods for the detection of decomposition be continued.

Approved.

FOOD PRESERVATIVES.

It is recommended—

(1) That further collaborative study be devoted to the process of removing benzoic acid from ketchup with the aid of chloroform.

Approved.

(2) That further collaborative study be devoted to the process of subliming benzoic acid, in order that optimal temperature and pressure conditions may be established.

Approved.

(3) That the official method for the determination of benzoic acid in other products than ketchup[1] be compared with methods involving the sublimation process.

Approved.

(4) That, as soon as possible, the sublimation process be applied to the determination of salicylic acid and to that of saccharin (in food samples into which these substances have been introduced), and that suitable procedures for their determination be devised.

Approved.

COLORING MATTERS IN FOODS.

It is recommended that the work planned for next year be conducted with the view to bringing the work recommended last year[2] to a definite conclusion.

Approved.

METALS IN FOODS.

It is recommended—

(1) That further study be made of methods for the determination of arsenic in foods.

Approved.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 128.
[2] *This Journal*, 1926, 9: 84.

(2) That the thiocyanate method for lead[1] be further studied.

Approved.

(3) That study of methods for the determination of copper and of zinc be continued.

Approved.

ZINC IN DRIED EGGS.

No report was submitted.

FRUITS AND FRUIT PRODUCTS.

WATER IN GRAPE JUICE.

It is recommended that, since the modified tentative method for the determination of added water in white grape juice[2] is sound in principle and entirely applicable to juices found on the market, no further work be done on the subject.

Approved.

FRUIT ACIDS.

It is recommended that work on fruit acids be continued.

Approved.

ASH IN FRUIT PRODUCTS.

The committee recommends—

(1) That methods proposed by the referee for the determination of calcium and magnesium in the ash of fruit products be subjected to collaborative study.

Approved.

(2) That the referee continue his studies on the determination of other ash constituents such as iron, aluminum, manganese, and chlorine.

Approved.

The committee repeats the recommendations made and approved last year[3] for further work on the several other topics under this title.

Approved.

CANNED FOODS.

No report was submitted.

The committee repeats the recommendations made and approved last year[4].

Approved.

VINEGARS.

It is recommended—

(1) That methods for total and for soluble ash be further studied.

Approved.

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[1] *This Journal*, 1926, 9: 366.
[2] *Ibid.*, 38.
[3] *Ibid.*, 85.
[4] *Ibid.*, 86.

(2) That the subject of phosphoric acid in vinegars be studied with the view to possible substitution of a method for total phosphoric acid for the present methods for soluble and insoluble phosphoric acids[1].

Approved.

(3) That the present tentative method for the determination of non-volatile reducing substances[2] be adopted as an official method (final action). (First action was taken in 1923.)

Approved.

(4) That the present tentative method for the determination of volatile reducing substances[2] be adopted as an official method (final action). (First action was taken in 1923.)

Approved.

(5) That the method for the determination of glycerol[3] be further studied.

Approved.

(6) That the method for polarization of vinegars[4] be further studied.

Approved.

(7) That methods for the determination of sulfates in vinegars[4] be further studied.

Approved.

FLAVORS AND NON-ALCOHOLIC BEVERAGES.

It is recommended—

(1) That the Folin and Denis rapid colorimetric method for the determination of vanillin in vanilla extract and its imitations, as described in the report of the referee[5], be adopted as an official method (final action).

The committee approves this recommendation and further recommends that this method be designated in *Methods of Analysis* as "Colorimetric"; and that the title of the present official method for Vanillin and Coumarin[6] be amended to read "Vanillin and Coumarin, Gravimetric.—Official".

Recommendations of the referee and of the committee approved.

(2) That the polariscopic method for the determination of oils of lemon, orange, and lime in corn oil, cottonseed oil, peanut oil and mineral oil, as described in the report of the referee this year (see p. 43), be adopted as a tentative method.

Approved.

(3) That the steam distillation method for the determination of essential oils in non-alcoholic flavors, as described in the report of the referee for 1925[7], be further studied.

Approved.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 325-6.
[2] *Ibid.*, 326.
[3] *Ibid.*, 327.
[4] *Ibid.*, 329.
[5] *This Journal*, 1926, 8: 688.
[6] *Methods of Analysis, A. O. A. C.*, 1925, 349.
[7] *This Journal*, 1926, 9: 450.

MEAT AND MEAT PRODUCTS.

It is recommended—

(1) That the present tentative method for the determination of nitrites[1] be referred to the Referee on Waters, Brine, and Salt for consideration and appropriate action.

Approved.

(2) That the present official method for the determination of total nitrogen[2] be amended to read, last clause, as follows:—"in the Kjeldahl-Gunning-Arnold method for one hour after the mixture has become colorless".

The committee does not approve this recommendation at this time because it is required that collaborative experience be secured before such action is taken.

Action of committee approved.

(3) The committee recommends that the method for the estimation of added water in sausage and other meat products, as outlined by the referee this year and suggested by him as a tentative method, be not adopted this year but held for further consideration.

Action of committee approved.

(4) The committee recommends that the suggestion of the referee for the deletion of the present tentative methods for the determination of soluble and insoluble nitrogen[3]; for coagulable nitrogen (**27**); for proteose, peptone, and gelatin nitrogen (**28**); for meat bases (**29**); for amino nitrogen (**31, 32, 33**, and **34**); for total soluble phosphorus (**35**); for the separation of soluble and insoluble phosphorus (**36**); and for soluble phosphorus in blood, brains, and glandular organs (**37**) be not adopted this year pending further consideration of the possible effects of such deletions and other aspects of the question.

Action of committee approved.

SEPARATION OF MEAT PROTEINS.

No report was submitted.

GELATIN.

It is recommended—

(1) That further study be made of the method of preparation of the sample by ashing as compared with that of hydrolysis.

Approved.

(2) That the determination of copper be studied for the purpose of developing a more satisfactory method than is now available.

Approved.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 240

[2] *Ibid*., 237.

[3] *Ibid.*, 243-4

(3) That the precipitation of zinc in formic acid solution be considered in further studies of methods for determining this metal.

Approved.

CACAO PRODUCTS.

It is recommended—

(1) That the Lepper-Waterman method for the determination of fat in cacao products[1] be adopted as an official method (final action).

Approved.

(2) That the official method for the determination of fat in cacao products[2] be deleted (first action).

Approved.

(3) That the modification of the old official method, as described by the referee this year and published previously[3], be adopted as a tentative method for the determination of fat in cacao products.

Approved.

(4) The committee repeats the recommendations made and approved last year[4] for further work on cacao products.

Approved.

MICROSCOPICAL METHODS.

No report was submitted.

CRUDE FIBER.

No report was submitted.

CACAO BUTTER.

No report was submitted.

CEREAL FOODS.

FLOUR.

It is recommended—

(1) That the method for sampling flour[5] adopted as tentative last year be subjected to collaborative study, as suggested by the associate referee.

Approved.

(2) That the present official vacuum oven method for the determination of moisture in flour[6] be dropped.

Approved.

(3) That the vacuum oven method for the determination of total solids and moisture (indirect method) in flour[7], be adopted as official (final action).

Approved.

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[1] *This Journal*, 1925, 8: 706; 1926, 9. 46.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 345.
[3] *This Journal*, 1926, 9: 468.
[4] *Ibid*, 95.
[5] *Ibid.*, 1926, 9: 39.
[6] *Methods of Analysis, A. O. A. C.*, 1925, 225.
[7] *This Journal*, 1926, 9: 39.

(4) That the routine air-oven method for the determination of total solids and moisture (indirect method) in flour[1] be adopted as official (first action); and that the word "routine" be deleted from the title.

Approved.

(5) That no associate referee on moisture in flour be designated for the coming year since these methods are now in satisfactory condition.

Approved.

(6) That the associate referee continue studies on rapid methods for the determination of ash in flour, including the alundum method and the oxygen-acetate method[2].

Approved.

(7) That the associate referee should carefully study the nature and kind of losses occurring when ash is fused.

Approved.

(8) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour[1], be adopted as an official method (final action).

Approved.

(9) That the method for the determination of lipoids and lipoid phosphoric acid (P_2O_5) in flour[1] be adopted as official (final action).

Approved.

(10) That the acid hydrolysis method for the determination of fat in flour[3] be adopted as an official method (first action).

Approved.

(11) That the modified Kerr-Sorber method for the determination of unsaponifiable matter[4] be adopted as a tentative method for the determination of the unsaponifiable matter in the fat of flour and subjected to further collaborative study.

This is the recommendation of the general referee and differs from that of the associate referee.

In view of the fact that the modified Kerr-Sorber method is recommended as tentative only and is to be subjected to further study, the committee approves the recommendation of the general referee.

Action of the committee approved.

(12) That the study of methods for the determination of glutenin in flour be continued and that the associate referee subject the Blish-Sandstedt[5] and "barium hydroxide" methods to collaborative study.

Approved.

(13) That the method submitted by the associate referee (see p. 33) be approved as a tentative method for the determination of the hydrogen-ion concentration of flour.

Approved.

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[1] *This Journal*, 1926, 9: 40.
[2] *Cereal Chem.*, 1926, 3. 222.
[3] *Ibid.*, 41
[4] *Ibid.*, 1925, 8: 441.
[5] *Cereal Chem.*, 1925, 2: 57.

(14) That collaborative studies on the determination of hydrogen-ion concentration of flour be continued, and that the collaborators be provided with standard buffer solution, to which their set-up shall be standardized before beginning the examination of samples.

Approved.

(15) That the associate referee give attention to the possible use of the quinhydrone electrode in this connection.

Approved.

(16) That the study of methods for the determination of gluten in flour be continued.

Approved.

(17) That the study of methods for the determination of the diastatic value of flour be continued and that this investigation include (a) a determination of the optimum hydrogen-ion concentration for the action of the diastase, (b) the influence of fermentation on this action, (c) the influence of proteolytic activity, and (d) methods of analysis.

Approved.

(18) That work on the detection and estimation of flour-bleaching chemicals be continued with special attention given to the determination of chlorine in chlorine-treated flours.

Approved.

(19) That the study of methods for the determination of starch in flour be continued and that this study include the method proposed by the associate referee and also the recently proposed modification of the diastase method as suggested by Hartmann and Hillig.

Approved.

(20) That consideration be given to the factors for the conversion of the percentages of nitrogen into terms of protein in wheat, wheat bran, wheat endosperm, and wheat embryo as suggested by Jones[1].

Approved.

BAKED CEREAL PRODUCTS.

It is recommended—

(1) That collaborative study of the tentative method for the preparation of sample of bread[2] be continued.

Approved.

(2) That the tentative method for the determination of total solids of an entire loaf of bread be further studied.

Approved.

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[1] *Cereal Chem.*, 1926, 3: 194.

[2] *This Journal*, 1926, 9: 42.

(3) That the tentative method for the determination of total solids of the air-dried ground sample[1] be further studied.

Approved.

(4) That studies of the 130°C. air-oven and other rapid methods for the determination of total solids in an entire loaf of bread be continued.

Approved.

(5) That the method for the determination of ash in baked cereal products[1] be adopted as official (final action).

Approved.

(6) That the method for the determination of protein in baked cereal products[1] be adopted as official (final action).

Approved.

(7) That comparative studies of the methods for the determinations of lipoids (as directed for alimentary pastes) and of fat in bread be continued.

Approved.

(8) That the study of methods for the carrying out of experimental baking tests be continued.

Approved.

ALIMENTARY PASTES.

It is recommended—

(1) That the tentative method for taking and preparing a sample of alimentary paste for analysis[2] be studied collaboratively.

Approved.

(2) That the tentative method for the determination of total solids and moisture (indirect method)[2] be studied collaboratively.

Approved.

(3) That the study of the air-oven method for the determination of total solids in alimentary pastes be continued.

Approved.

(4) That the method for the determination of ash in alimentary pastes[3] be made official (final action).

Approved.

(5) That the method for the determination of chlorides in ash as sodium chloride[3] be made official (final action).

Approved.

(6) That the method for the determination of organic and ammoniacal nitrogen in alimentary pastes[3] be made official (final action).

Approved.

(7) That the method for the determination of protein in alimentary pastes[4] be adopted as official (final action).

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[1] *This Journal*, 1926, 9: 42.
[2] *Ibid.*, 43.
[3] *Methods of Analysis, A. O. A. C.*, 1925, 232.
[4] *This Journal*, 1926, 9. 44.

Approved.

(8) That the method for the extraction and identification of added color in alimentary pastes[1] be made official (final action).

Approved.

(9) That the acid hydrolysis method for the determination of fat in flour[2] be adopted as a tentative method for the determination of fat in alimentary pastes and subjected to further collaborative study.

Approved.

(10) That the method for the determination of lipoids and lipoid phosphoric acid (P_2O_5)[3] be studied collaboratively.

Approved.

(11) That the modified Kerr-Sorber method for the determination of unsaponifiable matter[4] be adopted as a tentative method for the determination of the unsaponifiable matter in the fat of alimentary pastes and subjected to further collaborative study.

Approved.

(12) That the associate referee study the application to alimentary pastes of the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour[4].

Approved.

REPORT OF THE REPRESENTATIVES OF THE A. O. A. C. ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL[5].

It is the purpose of the representatives to report such investigations as are being actively conducted under the auspices of the Institute, so that this association may be in a position through the Institute's president, W. C. O'Kane, Durham, N. H., to learn where and by whom the work is being done.

There follows a list of the organizations which are taking advantage of the plan of the Institute, in accordance with which they are meeting the expenses incident to research on their products, to ascertain their direct or indirect value in combating pests or troubles which interfere with the normal output of farm products.

American Association of Nurserymen: Crown gall.

Bayer Company· Penetration of seed coats by seed-borne parasites, and thallium and mercury compounds as insecticides and rodenticides.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 233.
[2] *This Journal*, 1926, 9: 41.
[3] *Ibid.*, 40.
[4] *Ibid.*, 1925, 8: 441.
[5] Presented by J. B. Smith.

Copper refiners—Balbach Metals Corporation, Goldsmith Brothers Smelting and Refining Company, and Nichols Copper Company: A fundamental study of the value of copper compounds to agriculture.

B. G. Pratt Company: The merits of Scalecide.

Quaker Oats Company: Furfural derivatives and the value of a promising wound dressing for plants.

Standard Oil Company of Indiana: Emulsified spraying oils and cattle sprays and household insecticides.

Standard Oil Company of New Jersey: The usefulness of Flit for eliminating household and livestock insects, borers, ground animals, etc.

By consultation with those who are conducting these researches, many of which are of a fundamental nature, those who are particularly interested may get early information concerning the lines of investigation which are underway.

The following papers on the researches of the Institute have been published during the year:

Tisdale, L. E.—Colloidal sulphur: preparation and toxicity, Crop Protection Digest No. 8, and Ann. Mo. Bot. Gard. Vol. 12.

Young, H. C.—Colloidal sulphur as a spray material. Crop Protection Digest No. 7, and Ann. Mo. Bot. Gard. Vol. 12.

Ricker, A. J., and Muncie. J. H.—Suggestions on the preparation of apple grafts. Bull. No. 9, Crop Protection Institute.

Any scientific member of this association who has not joined the Institute may become a scientific member by conferring with Paul Moore, Secretary of Crop Protection Institute, National Research Council, Washington, D. C., and by paying one dollar annually.

An idea of the breadth of the Institute may be gained by one of its purposes, namely: to further co-operation among scientific workers and the producers of chemicals; the manufacturers of insecticides, fungicides, and other similar materials; the manufacturers of appliances required for their use; and the manufacturers, growers, packers, and shippers of the foregoing and of plant, animal, and other products.

BURT L. HARTWELL,
Approved. H. J. PATTERSON.

REPORT OF THE SECRETARY-TREASURER.

By W. W. SKINNER (Bureau of Chemistry, Washington, D. C.).

The work of the office of Secretary-Treasurer has proceeded satisfactorily because of its effective and efficient handling by Miss Lapp.

During the year E. M. Bailey was appointed to fill the vacancy on the Committee of Recommendations of Referees and also that on Subcommittee C caused by the death of Mr. Doolittle. L. E. Warren was appointed to fill the vacancy on Sub-committee B due to the transfer of Dr. Bailey.

It was necessary to make several reappointments in the list of referees and associate referees, but all vacancies were eventually filled except the refereeship on Starch Conversion Products under Sugars and Sugar Products. The field of those experienced in this line of work is so limited that the possibilities were soon exhausted. In this connection, however, it might be stated that it is encouraging to receive letters now and then from those who desire to collaborate on special work.

L. H. McRoberts, Bismarck, N. Dak., was appointed as Referee on Ice Cream, to take the place of A. C. Dahlberg, and H. A. Lepper took the place of H. W. Redfield. Both of these resignations were caused by ill health. P. L. Gowen was succeeded as General Referee on Fruits and Fruit Products by H. J. Wichmann, and Raymond Hertwig by F. C. Blanck as General Referee on Cereal Foods. C. O. Swanson succeeded E. L. Tague of Manhattan, Kans., as Referee on the Diastatic Value of Flour; G. C. Spencer succeeded Armin Seidenberg as Referee on Chlorine in Bleached Flour; and L. E. Warren succeeded A. G. Murray as Referee on Apomorphine.

The usual number of inquiries relating to different subjects and methods were received, the majority of which were referred to the proper referees. All correspondence tends to show a marked increase in interest in the work of the association and especially *Methods of Analysis*. The report of the Chairman of the Board of Editors has emphasized this point in showing the details of the sales of this book.

Not all criticism, however, is favorable. One correspondent censures the committee for republishing the Chapter on Distilled Liquors substantially as it was in the 1920 edition. The answer was to the effect that there was even some doubt about including this chapter and the chapters on beers and wines because of their small interest to agricultural chemists, but that before another edition they would be brought up to date or eliminated.

At the meeting of the Executive Committee last evening, the Secretary-Treasurer reported cash on hand of $1,056.71 and suggested the advisability of investing $1,000.00 of said funds in some interest-bearing security. The committee then authorized the treasurer to invest this amount in some easily convertible security.

The Executive Committee approved the continued cooperation with the American Public Health Association in the preparation and publication of the Standard Methods for Milk Analysis and appointed the Chairman of the Board of Editors and the General Referee on Dairy Products a committee with power to act in this matter.

The Executive Committee approved the following new associate refereeships: On methods for the determination of proteins in milk products, on methods of analysis of flourine compounds, on methods for the determination of the less common metals in soils, on methods for the determination of the less common metals in plants, and on qualitative tests for dairy products.

The passing of our worthy colleague and past president, R. E. Doolittle, has been commented upon by others, and proper resolutions will be prepared and presented to the association. A suitable biographical sketch will be published in *The Journal*. The great work that he accomplished for the A. O. A. C. cannot be over-emphasized. The association honored him and itself by electing him president two years ago.

Mr. Doolittle had a fine sense of the value of the leaven of play in daily work, and so he would have enjoyed this meeting, which has been unusual in that some sessions of levity have been injected into the usual business proceedings. Mr. Chairman, it is suggested that this little group of friends of Mr. Doolittle rise and stand for a moment in silence in honor of his memory (period of silence).

The financial statement shows little change. Sixty-one institutions have paid dues compared with 69 last year, but this difference is accounted for by the fact that bills were not sent out until late in the year and several have paid their dues since the books were closed, October 1, 1926.

The detailed statement will be found on p. 53.

It has been requested that the following invitation be presented to the association:

Dear Doctor Skinner:

On behalf of the Organizing Committee of the International Congress of Soil Science an invitation is extended to the Association of Official Agricultural Chemists to participate in the meetings of the Congress to be held in Washington, D. C , on June 13 to 22, 1927, and it is requested that the association appoint a committee to cooperate with the Organizing Committee in making the necessary arrangements and carrying out the plans for the meetings of the Congress.

Very truly yours,

(Signed) OSWALD SCHREINER,
Chairman, Washington Section.

W. H. MacIntire: The Organization Committee requested me to say a word in connection with this invitation. This gathering of international soil scientists, to be held in Washington next June, has been designated as the First International Congress of Soil Science. Strictly speaking, it is the fifth, for we have had four meetings on somewhat similar lines in different cities in Europe. As an outgrowth of those meetings, the International Congress was formed, and it will convene annually. America received the honor of having the president through the selection of Dr. J. G. Lipman.

This Congress has been accorded official recognition, and on that authority the Secretary of State has issued invitations to all the foreign governments. Delegates are expected from all these countries, and their expenses will be paid and provided for by their respective governments. We propose to utilize this opportunity to show these representatives something of the life and the industries of America. We also propose to take 300 of the officials designated as representatives across the continent and to show them the wonders of California. We shall have a special train for which $160,000 will be raised. One-half of that amount has already been pledged. The meeting will be for a period of 10 days. Invitation is extended to members of this association to join the transcontinental journey, which will first go to the southern part of the country and then up the Pacific coast. Of course, we are unable to care for the expenses of our own people; they must be borne individually or by the several organizations, but we have already enough people to fill two special trains. Unusual entertainment will be provided, and we believe that this is a splendid opportunity for the scientists of this country to meet with the scientists of other countries. On the request of this Committee on Organization, Mr. President, I move that such a committee as requested be appointed from this association. I presume it will be designated the Committee on Collaboration, and that its function will be to extend greetings to the Congress when it convenes here.

It was moved, seconded, and carried that a committee of three be appointed, on which the incoming president will be an ex-officio member, to represent this association at the International Congress of Soil Science next June.

ANNOUNCEMENT OF THE FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE.

In accordance with the decision of the Fourth International Conference of Soil Science, which met in Rome in May, 1924, the first meeting of the International Congress of Soil Science, then organized, will convene on June 13, 1927, in Washington, D. C. The congress will be followed by a field excursion to visit the various important soil belts in the country. Opportunity will also be given to the delegates to acquaint themselves with various agricultural industries, some of the leading agricultural experiment stations, and in general with the agricultural resources of the United States.

The association is made up of six international commissions, which are given with the American representatives, as follows:

I. *Commission on Soil Physics.*—Dr. C. Davis, Bureau of Soils, Washington, D. C.

II. *Commission on Soil Chemistry.*—Dr. M. M. McCool, E. Lansing, Mich.

III. *Commission on Soil Bacteriology*—Dr S. A. Waksman, New Brunswick, N. J.

IV. *Commission on Soil Fertility.*—Prof D. R. Hoagland, Berkeley, Calif.

V. *Commission on Nomenclature, Classification, and Cartography*—Dr. C. F. Marbut, Bureau of Soils, Washington, D. C.

VI. *Commission on the Application of Soil Science to Land Cultivation*—Dr S. H. McCrory, Bureau of Agricultural Engineering, Washington, D. C.

Each commission is now working on the preparation of its own program. Some of the sessions will be devoted to the congress as a whole or to combined meetings of more than one commission, while a number of sessions (5 to 8) will be devoted to the special sessions of each commission.

The program of each commission will consist of papers presented by invitation by outstanding investigators in the respective fields, and of papers presented by various workers in the different branches of soil science, members or non-members. Titles of the papers to be presented and brief abstracts in English, French, and German should be sent on or before December first to the respective chairman, to the American representative of the commission where the paper is to be presented, or to the president of the association, who will have the paper forwarded to the chairman of the corresponding commission.

This congress will bring together in this country, for the first time in its history, all those that are interested in the different problems of soil classification, soil analysis, fertilization, and treatment, as well as the relation of the soil to plant growth. Extensive exhibits of various soil types (monolithic columns, in respective horizons) from Europe and America, apparatus used in soil analyses, soil microflora and microfauna, etc., will be held during the congress.

Dr. J. G. Lipman of New Brunswick, N. J., is president, and Dr. D. J. Hissink of Groningen, Holland, is secretary.

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS.

This committee respectfully submits the following report covering the proceedings of the Joint Committee on Food and Drug Definitions and Standards during the past year:

The committee held one meeting, the 30th since its organization, during the two weeks beginning January 18, 1926. Hearings were held on several important subjects, and final action was taken on schedules of definitions and standards that have been under consideration for some time past. A conference was held with manufacturers of alimentary pastes, chiefly with reference to the character of raw material entering into the manufacture of macaroni and the use of added coloring. After considerable discussion on the part of the committee a further revision was made of the schedule that was adopted in tentative form during the meeting held early in 1925. Definitions were approved for the following terms: alimentary pastes, egg alimentary pastes, plain alimentary pastes, noodles, egg noodles, and water noodles. The moisture standard of 13 per cent applicable to all alimentary pastes was reaffirmed.

An afternoon session was devoted to a conference with manufacturers and State food officials on the subject of so-called process cheese. Much valuable information was obtained relative to the moisture and fat content of these products, the use of emulsifiers and other added substances, and methods of labeling. The committee decided after some discussion of these subjects to postpone further consideration until a later meeting. The flour milling industry was well represented at a hearing held during the second week's session. The chief subject under discussion related to the proposed change in the standard for moisture. After a thorough examination of the results of investigations that have been conducted in recent years relative to the merits of various methods for determining moisture, the committee decided to recommend a revision of the present definition and standard for flour, in form as follows:

Flour is the fine, clean, sound product made by bolting wheat meal. It contains not more than fifteen per cent (15%) of moisture[1], not less than one and twenty-five hundredths per cent (1.25%) of nitrogen, not more than one per cent (1%) of ash, and not more than one-half per cent (0.5%) of fiber.

This definition and standard has been approved by the Department of Agriculture and promulgated in Food Inspection Decision 204, issued under date of August, 1926.

The committee devoted considerable time to a continued discussion of the fruit products schedule that was adopted in tentative form during the last meeting held in the previous year. The schedule was thoroughly revised and finally adopted in form as follows:

1. *Fruit* is the clean, sound, edible, fleshy fructification of a plant and is characterized by its sweet, acid, and/or ethereal flavor.
2. *Fresh fruit* is fruit which has undergone no material change other than ripening since the time of gathering.
3. *Dried fruit* is the clean, sound product resulting from the evaporation of the greater portion of the water from properly prepared fresh fruit.

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[1] By "moisture" is meant the loss in weight resulting from drying in accordance with the vacuum method of the Association of Official Agricultural Chemists. The moisture limit of 15 per cent, thus determined, is regarded as equivalent to the former moisture limit of 13.5 per cent, as determined by the water-oven method.

[2] *This Journal*, 1926, 9: 39.

(*a*) The term "sundried" is commonly used to designate the product dried without the use of artificial heat.

(*b*) The terms "evaporated" and "dehydrated" are commonly used to designate the product dried by the use of artificial heat.

4. *"Cold-pack" fruit* is the clean, sound product obtained by packing, in a suitable container, properly prepared fresh fruit, with or without the addition of sugar (sucrose), and maintaining it at a temperature sufficiently low to insure its preservation.

5. *Canned fruit* is the clean, sound product made from properly prepared fresh fruit, with or without water and/or sugar (sucrose),

(*a*) By processing in a suitable, hermetically sealed container, or

(*b*) By heating and packing in a suitable container which is then hermetically sealed.

6. *Preserve, fruit preserve, jam, fruit jam,* is the clean, sound product made by cooking to a suitable consistency properly prepared fresh fruit, "cold-pack" fruit, canned fruit, or a mixture of two or of all of these, with sugar (sucrose) or with sugar and water. In its preparation not less than forty-five (45) pounds of fruit are used to each fifty-five (55) pounds of sugar (sucrose).

A product in which the fruit is whole or in relatively large pieces is customarily designated a "preserve" rather than a "jam."

7. *Glucose fruit preserve, corn sirup fruit preserve, glucose fruit jam, corn sirup fruit jam,* is the clean, sound product made by cooking to a suitable consistency properly prepared fresh fruit, "cold-pack" fruit, canned fruit, or a mixture of two or of all of these, with glucose or corn sirup. In its preparation not less than forty-five (45) pounds of fruit are used to each fifty-five (55) pounds of glucose or corn sirup.

8. *Fruit butter*[1] is the sound product made from fruit juice and clean, sound, properly matured and prepared fruit, evaporated to a semisolid mass of homogeneous consistence, with or without the addition of sugar and spices or vinegar, and conforms in name to the fruit used in its preparation.

9. *Glucose fruit butter, corn sirup fruit butter,* is a fruit butter in which glucose, or corn sirup, is used in place of sugar (sucrose).

10. *Jelly, fruit jelly,* is the clean, sound, semisolid, gelatinous product made by concentrating to a suitable consistency the strained juice, or the strained water extract, from fresh fruit, from "cold-pack" fruit, from canned fruit, or from a mixture of two or of all of these, with sugar (sucrose).

11. *Glucose fruit jelly, corn sirup fruit jelly,* is the clean, sound, semisolid, gelatinous product made by concentrating to a suitable consistency the strained juice, or the strained water extract, from fresh fruit, from "cold-pack" fruit, from canned fruit, or from a mixture of two or of all of these, with glucose or corn sirup.

12. *Citrus fruit marmalade* is the clean, sound, jelly-like product made from the properly prepared juice and peel, with or without the pulp, of fresh citrus fruit, of canned citrus fruit, or of a mixture of these, by cooking with water and sugar (sucrose). It contains, embedded in the mass, pieces of the fruit peel, with or without portions of the pulp of the fruit.

These definitions and standards have been approved by the Department of Agriculture and promulgated in Food Inspection Decision 203, issued under date of August, 1926.

Further attention was given to the subject of Dutch-process or "alkalized" chocolate and cocoa, with the result that a definition was finally approved and recommended for adoption, as follows:

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[1] This item has not been revised.

Dutch-process chocolate, "alkalized chocolate", and Dutch-process cocoa, "alkalized cocoa", are modifications, respectively, of chocolate and cocoa, in that in their manufacture an alkali carbonate, or other suitable alkaline substance, has been employed. In the preparation of these products not more than three (3) parts by weight of potassium carbonate, or the neutralizing equivalent thereof in other alkaline substances, are added to each one hundred (100) parts by weight of cacao nibs. The finished products conform to the standards for chocolate and cocoa, respectively, due allowance being made for the kind and amount of alkaline substance added.

This definition and standard was promulgated by the Department of Agriculture in Food Inspection Decision 202.

A joint conference was held with representatives of the spice trade and members of the Bureau of Chemistry, chiefly with reference to standards for Saigon cassia, marjoram, Pedang mace, American sage, and coriander seed. The ash standards of these various products were given consideration, but no final action was taken. After several years of continuous efforts, a final draft was made of the definitions for meat and meat products, and the schedule was recommended for adoption in the following form:

a. MEATS.

1. *Flesh* is any clean, sound, edible part of the striated muscle of an animal. The term "animal," as herein used, indicates a mammal, a fowl, a fish, a crustacean, a mollusk, or any other animal used as a source of food.
2. *Meat*[1] is the properly dressed flesh derived from cattle, from swine, from sheep, or from goats, sufficiently mature and in good health at the time of slaughter, but is restricted to that part of the striated muscle which is skeletal or that which is found in the tongue, in the diaphragm, in the heart, or in the esophagus, and does not include that found in the lips, in the snout, or in the ears; with or without the accompanying and overlying fat, and the portions of bone, skin, sinew, nerve, and blood vessels which normally accompany the flesh and which may not have been separated from it in the process of dressing it for sale.
3. *Fresh meat* is meat which has undergone no substantial change in character since the time of slaughter.
4. *Beef* is meat derived from cattle nearly one year of age, or older.
5. *Veal* is meat derived from young cattle one year or less of age[2].
6. *Mutton* is meat derived from sheep nearly one year of age, or older.
7. *Lamb* is meat derived from young sheep one year or less of age[2].
8. *Pork* is meat derived from swine.
9. *Venison* is flesh derived from deer.

b. MEAT BY-PRODUCTS.

1. *Meat by-products* are any clean, sound, and properly dressed edible parts, other than meat, which have been derived from one or more carcasses of cattle, of swine, of sheep, or of goats, sufficiently mature and in good health at the time of slaughter.

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[1] The term "meat" when used in a qualified form, as, for example, "horse meat", "reindeer meat", "crab meat", etc., is then, and then only, properly applied to the corresponding portions of animals other than cattle, swine, sheep, and goats.

[2] Minimum limits governing the age or the weight or both of these have been fixed by certain States and municipalities in the case of calves and lambs to be slaughtered for meat.

c. PREPARED MEATS.

1. *Prepared meat* is the clean, sound product obtained by subjecting meat to a process of comminuting, of drying, of curing, of smoking, of cooking, of seasoning, or of flavoring, or to any combination of such processes.

2. *Cured meat* is the clean, sound product obtained by subjecting meat to a process of salting, by the employment of dry common salt or of brine, with or without the use of one or more of the following: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, a sirup, honey, spice.

3. *Dry salt meat* is the prepared meat which has been cured by the application of dry common salt, with or without the use of one or more of the following: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, a sirup, honey, spice; with or without the injection into it of a solution of common salt to which may have been added one or more of the following: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, a sirup, honey.

4. *Corned meat* is the prepared meat which has been cured by soaking in, with or without injecting into it, a solution of common salt, with or without one or more of the following, each in its proper proportion: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, a sirup, honey, and with or without the use of spice.

5. *Sweet pickled meat* is the prepared meat which has been cured by soaking in, with or without injecting into it, a solution of common salt with sugar, a sirup, and/or honey, together with one or more of the following, each in its proper proportion: Sodium nitrite, sodium nitrate, potassium nitrate, and with or without the use of spice

6. *Dried meat* is the clean, sound product obtained by subjecting fresh meat or cured meat to a process of drying, with or without the aid of artificial heat, until a substantial portion of the water has been removed.

7. *Smoked meat* is the clean, sound product obtained by subjecting fresh meat, dried meat, or cured meat to the direct action of the smoke either of burning wood or of similar burning material.

8. *Canned meat* is fresh meat or prepared meat, packed in hermetically sealed containers, with or without subsequent heating for the purpose of sterilization.

9. *Hamburg steak*, "*Hamburger steak*", is comminuted fresh beef, with or without the addition of suet and/or of seasoning.

10. *Potted meat, deviled meat*, is the clean, sound product obtained by comminuting and cooking fresh meat and/or prepared meat, with or without spice, and is usually packed in hermetically sealed containers.

11. *Sausage meat* is fresh meat or prepared meat, or a mixture of fresh meat and prepared meat, and is sometimes comminuted The term "sausage meat" is sometimes applied to bulk sausage containing no meat by-products.

d. MEAT FOOD PRODUCTS.

1. *Meat food products* are any articles of food or any articles that enter into the composition of food which are not prepared meats but which are derived or prepared, in whole or in part, by a process of manufacture from any portion of the carcasses of cattle, swine, sheep, or goats, if such manufactured portion be all, or a considerable and definite portion, of the article, except such preparations as are for medicinal purposes only.

2. *Meat loaf* is the product consisting of a mixture of comminuted meat with spice and/or with cereals, with or without milk and/or eggs, pressed into the form of a loaf and cooked.

3. *Pork sausage* is chopped or ground pork, with or without one or more of the fol-

lowing: Herbs, spice, common salt, sodium nitrite, sodium nitrate, potassium nitrate, sugar, a sirup, water, vinegar; and may be fresh, dried, smoked, or cooked[1].

4. *Brawn* is the product made from chopped or ground and cooked edible parts of swine, chiefly from the head, feet, and/or legs, with or without the chopped or ground tongue.

5. *Head cheese, mock brawn,* differs from brawn in that other meat and/or meat by-products are substituted, in whole or in part, for corresponding parts derived from swine.

6. *Souse* is the product consisting of meat and/or meat by-products; after cooking, the mixture is commonly packed into containers and covered with vinegar.

7. *Scrapple* is the product consisting of meat and/or meat by-products mixed with meal or the flour of grain, and cooked with seasoning materials, after which it is poured into a mold.

Following the customary procedure, these definitions and standards were approved by the Department of Agriculture and published in Food Inspection Decision 205.

Some of the definitions were either revoked or revised and amended. Revisions and amendments were made in the definitions and standards for *malt vinegar* and *wine vinegar* in order to make them harmonize with the definition for cider vinegar that was adopted and published two years ago. The definition for *glucose, mixing glucose, confectioner's glucose,* was revised and simplified by omitting unnecessary stipulations, and in its present wording it is essentially a reaffirmation of the definition that has been in effect many years.

The definitions for *gluten flour, self-rising, "diabetic" food,* and *canned pea grades,* were recommended for revocation. A change was made in the definition for *sweetened condensed skimmed milk* whereby the standard for milk solids was lowered from 28 per cent to 24 per cent, and a minor change was made in the definition for *pasteurized milk.* The definitions for *blended milk* and *sterilized milk* were revoked. These revised and amended definitions and standards were approved and published by the Department of Agriculture in Food Inspection Decisions 198, 199, 200, and 201.

JULIUS HORTVET, E. M. BAILEY.
C. D. HOWARD,
Committee to Cooperate with Other Committees on Food Definitions.

Approved.

REPORT OF COMMITTEE ON SAMPLING.

In the 1925 report[2], this committee agreed (1) to undertake the preparation of complete bibliographies on and suggestions for the proper sampling of the various types of products, and (2) to undertake the preparation of an outline of work for consideration by the various referees.

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[1] The definition of other types of sausages is postponed for further consideration.
[2] *This Journal,* 1926, 9: 107. See also *This Journal,* 1925, 8: 287.

Owing to the vast amount of work involved in the above recommendation, it has not been possible for the committee to complete its assignment. However, substantial progress has been made and there are appended hereto five bibliographies covering the literature on sampling of soils and liming materials, tanning materials and leathers, saccharine products, foods, and drugs.

The committee believes that the work outlined at the 1925 meeting can be completed during the coming year and, therefore, recommends its continuation.

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F. W. Zerban,
A. J. Patten,
A. G. McCall,
R. W. Frey,
J. W. Sale,
A. E. Paul.

Committee on Sampling.

Approved.

MEMORANDUM ON SAMPLING OF SOILS.

By A. G. McCall and R. R. McKibbin (Laboratory for Soil Investigations, University of Maryland).

It is apparent that even when a large sample of soil is collected in the field it is difficult to obtain one fully representative of the area under study, and unless great care is taken in handling the sample in the laboratory the results of analysis may have little value.

If it were possible to have homogeneous soils over wide areas, with no gravel or organic matter of any size scattered through them, the difficulties of getting a representative sample would be minimized. As it is, however, soils vary widely in their content of sand and gravel and stones, and in the amount of undecomposed or semidecomposed organic debris.

No attempt is made to include the sticks and stones in the chemical analysis of a soil. However, the coarse particles are weighed separately, and the analytical results are expressed in terms of the entire soil and not in terms of the fine earth separate only, although the latter is the only portion included in the chemical analysis.

One of the early workers in the field, Johnston (10)[1], directs that the sample be oven-dried in its natural state at the temperature of boiling water and then that "an unweighed portion of the soil in its natural state be introduced into five or six ounces of boiling distilled water in a flask and kept at a boiling temperature with occasional shaking for a quarter of an hour". Presumably this treatment was designed to get all the soil constituents into solution.

About sixty years ago Wolff (19), a German worker, directed that the air-dried soil be freed from stones, passed through a 3 mm. mesh sieve, and kept in glass bottles before it was used for chemical analysis.

The German worker Schone[2] defines "Feinerde" as the material passing through a 3 mm. mesh sieve, while Knops[3] defines it as the soil passing through a one-quarter millimeter mesh sieve. In 1875 the American, Burchard (2), gave the following directions as to the handling of a soil sample prior to chemical analysis: "A pound or two of the specimen is air-dried, carefully triturated in a porcelain mortar with a wooden pestle, and sifted through a screen, the meshes of which are 0.8 millimeter in diameter". He took 15 to 20 grams of the fine earth, dried at the temperature of boiling water, for mechanical analysis. For chemical analysis, apparently, the air-dried sifted soil was used.

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[1] Numbers used in text refer to bibliography

[2] Über Schlammanalyse und einen neuen Schlammapparat, p. 61 Berlin, 1867

[3] Die Bonitierung der Ackererde Leipzig, 1872.

In 1881 Laufer and Wahnschaffe[1] defined "Feinerde" or "Feinboden", that is material ready for chemical analysis, as soil that will pass through a 2 mm. mesh sieve.

The father of the Association of Official Agricultural Chemists, Wiley (17), directs the removal of all stones, gravel, and screening through a 1 or 2 mm. mesh sieve. He does not insist upon washing the coarse fractions.

Grandeau (5) gives the following complete directions for the handling of the sample when brought into the laboratory for analysis: "The container is examined to see whether the soil is dry or moist. If too moist, air-dry it; if dry in lumps, add a little distilled water, pulverize the lumps, and air-dry. The condition of the soil for spreading out to dry is best governed by the operator's experience

"Take one kilogram of air-dry soil and screen it; it is only that soil passing through Screen No. 1, i. e., apertures of one square millimetre, that is chemically analyzed. Calculations are always made to the weight of the unscreened sample."

Another French worker, Dehérain (3), directs that 200 grams of soil be placed on a 1 mm. mesh screen. The screen is shaken and thus most of the fine material runs through. The remaining part must be triturated gently in a mortar so as to make sure that all the concretions of fine soil are broken up and yet none of the coarser fractions are broken. Then the residue on the screen is weighed.

Yet another French worker, Guillin (6), gives the following directions: "2 kilograms of the soil should be placed on a brass plate and dried at 100°C. One kilogram of the dry soil is placed in a vessel with enough distilled water to cover it. After two hours the lumps and concretions have broken down and it is possible by screening to separate the finer soil from the coarser. The mixture of soil and water is now decanted on a wire screen having ten meshes to the centimeter, i. e., 1 mm. mesh, and 20 centimeters in diameter. With a wooden spatula the soil is moved about and a fine jet of distilled water played on it.

"Thus all the finer material goes through the screen and all the coarser remains on it. Only 500 cc. of water should be necessary per kilogram of soil.

"The fine and coarse separates are dried in porcelain evaporating dishes. Then the fine separate is triturated in a mortar and is used for chemical analysis. The coarse separate is weighed and the relative amounts of fine and coarse estimated. Express the analytical results in terms of the entire soil."

English methods of sampling soils are represented by the Rothamsted method. Hall (7) says:

> "When the samples reach the laboratory they are spread out on shallow trays to dry, which process may be accelerated by a gentle warmth, not exceeding 40°C. In dealing with stiff soils it is advisable to crumble all the lumps by hand while the earth is still somewhat moist.
>
> "When the whole is sensibly dry the stones are separated by a sieve having round holes 3 mm. in diameter; the material that does not pass the sieve is gently worked up in a mortar with a wooden pestle, care being taken not to break the stones, chalk, etc., but only to crush the lumps of earth. Finally, the material upon the sieve is roughly weighed and well washed in a stream of water till all the fine earth is gone, dried, picked over to free it from roots and stubble, and weighed as 'stones'. To get the proportion borne by the stones to the soil, the fine earth is also weighed, an addition being made of the weight lost by the stones in washing."

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[1] Untersuchungen des Bodens der Umgegend von Berlin—Abhandlungen zur geologischen Specialkarte von Preussen und den Thüringischen Staaten. Berlin, 1881 Bd III H. 2, p. 15.

The German investigator Mitscherlich (14) recommends passing the soil through a sieve with apertures 1.5 mm. square.

METHODS OF PROCEDURE DISCUSSED.

The following variations in procedure are noted:

1.—*Drying the soil.*
  (a) Most workers air-dry the soil, presumably at room temperature.
  (b) Hall suggests the application of gentle heat, but not higher than 40°C.
  (c) Guillin suggests drying the soil prior to screening at 100°C.

2.—*Screening wet or dry.*
  (a) The English and German custom seems to be to screen dry and then wash the coarse residue free of fine earth with water.
  (b) The French suggest washing the fine earth through the screen with water.
  (c) The American custom seems to be to screen dry and not wash the coarse residue.

3.—*Size of apertures in screens used.* This is the most disputed point: The size of aperture recommended varies from 0.25 mm. (Knops) to 3 mm.
  (a) The German and English workers prefer larger apertures than 1 mm. Commonly 3 mm. mesh screens are recommended.
  (b) The French and American workers prefer 1 mm. mesh screens.

4.—*Estimation of composition of soil.*
  The opinion of workers is unanimous that the fine earth separate should be used for chemical analysis and that the results should be expressed on the basis of the weight of the soil as a whole, that is, the weight of fine and coarse separates together.

While it is true that surface is all important in any consideration of the soil particles, yet it is by no means certain that it is the best procedure to pass the fine earth for analysis through a 1 mm. mesh screen rather than a larger meshed screen Modern ideas as to the soil solution emphasize the importance of the dynamic nature of soil equilibria. In making the total analysis of soil composition it is possible that the fine and coarse separates should be triturated, placed together, and thoroughly mixed, and the analysis made on the mixture.

It is believed that the practically universal acceptance of air-dry soil is justified, and that it is unnecessary to apply artificial heat.

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By R. W. Frey[1].

TANNING MATERIALS.

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[1] W. D. Edington gave assistance in compiling the bibliography.

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By F. W. Zerban.

Official instructions for the sampling of raw sugars, sirups, and molasses imported into the United States are found in Document No. 2470, U. S. Treasury Department, Division of Customs, Articles 3 to 22, reprinted in Circular 44 of the Bureau of Standards (1914). Essentially the same methods are used by the sugar trade in the United States; cf. C. A. Browne, A Handbook of Sugar Analysis, Chapter I, pages 3-14.

Methods of sampling raw sugars in different countries are described by Wiechmann in *Intern. Sugar J.*, 1917, **9**: 18–28.

Official methods of sampling prescribed in Germany are given in Frühling's Anleitung, 9th edition, by A. Rössing,—for raw sugars on page 126, for molasses on page 189, and for beets on page 242.

Sampling of the various saccharine materials produced in the manufacture of sugar from the beet and the cane is fully discussed in several books, such as

G. L. Spencer—Handbook for Cane-sugar Manufacturers and their Chemists.

G. L. Spencer.—Handbook for Chemists of Beet-sugar Houses and Seed-culture Farms.

Association of Hawaiian Sugar Technologists.—Chemical Control of Cane Sugar Factories.

H. C. Prinsen Geerligs.—Chemical Control in Cane Sugar Factories.

COMMENTS: In the judgment of this writer, the association is interested in the sampling of those products which appear in the trade, such as sugars, sirups (including glucose and honey), and molasses, but not in the methods of sampling intermediary sugar factory products. Practices in the sampling of the former products are well established. Some improvement could probably be effected in the sampling of liquid products, especially when the latter are contained in large storage tanks. Whether methods of sampling sugar cane and sugar beet should be taken up by the association is a debatable question.

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By F. C. BLANCK.

The trier compared with the wedge method of sampling tub butter. Ellenberg and Guthrie. *J. Dairy Sci.*, 1925, **8**: 80.

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The function of the chemical engineer in the biscuit and cracker baking industry. Lippert. *Chem. Met. Eng.*, 1920, **22**: 893.

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Sampling Committee Report 1923-24. *J. Oil and Fat Industries*, 1924, **1**: 46.

SAMPLING APPARATUS.

Triers for sampling flour. *This Journal*, 1925, **8**: 424.

New apparatus for investigating potatoes, grain, and their products. *Chem. App.*, 1916, **3**: 199.

SAMPLING DRUGS.

(Brief summary of literature consulted.)

By Arthur E. Paul.

The available material to be presented this year has been grouped and arranged in accordance with the following outline:

- *Crude drugs—bulk*
  - Roots, leaves, herbs, barks.
  - Seeds, berries.
  - Dry resins, gums.
  - Semi-solid resins, oleoresins.
- *Crude drugs—packages*
- *Pharmaceuticals*
  - Liquids.—Bulk, bottles.
  - Powders.—Bulk, packages.
  - Semi-solids.—Extracts, masses, plasters, ointments.
  - Pills.—Tablets.
  - Ampoules.

CRUDE DRUGS—BULK.

The first thing to be decided in sampling this class of products would seem to be the number of units to be selected from a given lot. It has recently been proposed that, for certain classes of foods, the number of units should equal, approximately, the square root of the number available. This seems, in the majority of instances, to be a suitable system. More specific suggestions are made in par. 7 of a report dated 5/26/19, prepared for the Central District by A. E. Paul, B. G. Hartmann, and H. L. Schultz, and in the report by H. L. Schultz, 7/19/17, Sampling of Import Foods.

ROOTS, LEAVES, HERBS, BARKS.

A marked step forward was taken by L. J. Schwartz in the development of methods for satisfactorily sampling this most perplexing group of substances. He presented his work during the Spring meeting, 1925, of the American Drug Manufacturers Association, New York. The important feature of his paper was a cut of a cylindrical trier, the end of which is provided with a cutting edge or with saw teeth. It may be equipped for either hand or electrical use. It removes a core 1¼ inches in diameter.

Certain limitations are mentioned by Schwartz, and it is pointed out that his device is not satisfactory for sampling heavy barks and roots, such as yohimbe, hydrangea, or gentian, nor for material baled under high pressure, such as Japanese insect flowers. In this connection, it is believed that in order to make the tool to meet the needs of various products, it should be provided with an assortment of tips. It is suggested that at least some of these tips be made of tool steel and that there be at least one tip with saw teeth set in the manner of an ordinary saw. This style might be satisfactory for heavy barks and thick roots as well as for solidly packed materials. It is considered that attention, on the committee's part, to this entire matter of sampling roots, barks, and herbs by means of this device, would be desirable.

Schwartz makes no suggestions in his paper relative to the number of cores to be taken from a single bale, but it would seem that the total sample should not be less than 500 grams. The number of cores from each bale would, therefore, vary with the number of bales to be sampled. This committee might profitably work out specific details for sampling lots consisting of varying numbers of units.

From this point, it would seem that the directions beginning on page 463 of the U. S. Pharmacopeia will apply. It is considered, however, that these directions, particularly I, are not clear and that they should be restated. Particular attention is called to the phrase "1 cm. or less in any dimension". If interpreted literally, it will lead to an incorrect conclusion, although consideration of II will, undoubtedly, correct the difficulty. Nevertheless, it is considered unfortunate that this vagueness exists.

SEEDS, BERRIES.

Relative to the number of units to be sampled, the general square-root principle is suggested, and the general U. S. Pharmacopeia directions mentioned above are quite applicable.

It seems to be the general opinion that these products are sampled most satisfactorily with a trier. Probably a series of triers should be employed in order to be generally usable. Useful suggestions are made in Central District's report on sampling, May 26, 1919, pages 15, 16, and 18. These may be applied preliminary to the U. S. Pharmacopeia details beginning on page 463.

DRY RESINS, GUMS.

The directions given in Central District's report on sampling, May 26, 1919, pages 19 and 20, agree very well with those given in Schultz's report of July 19, 1917, page 7, and it is believed that they may be considered sufficiently satisfactory to be used in connection with the U. S. Pharmacopeia directions.

SEMI-SOLID RESINS AND OLEO RESINS.

Attempts are made in both above mentioned reports, pages 14 and 7, respectively, to make useful suggestions. These appear to be the best available at this time, although in his report on crude drug sampling Schwartz indicates that promising work is in progress in connection with certain members of this group.

CRUDE DRUGS IN SMALL PACKAGES.

Crude drugs are frequently put up in compressed form in 1 pound packages. These packages may be the ultimate units, or they may consist of 16 one ounce packages. In any event, it would seem that the square-root rule should prevail, and that a representative portion of each be made up into a composite sample. This may then be treated according to the U. S. Pharmacopeia, beginning with page 463.

PHARMACEUTICALS.

LIQUIDS.

Liquid drugs are not usually put up in large containers, and particular attention need hardly be devoted to this class of product. If large, the containers ordinarily may be shaken, and a desired quantity drawn off; if small, an appropriate number of units may be taken. In its final report, it may be desirable for the committee to prepare a statement relative to the quantity of sample desired for analysis, and the number of units to be secured or sampled—for different classes of compounds—sizes of units, and size of shipment. Some desirable suggestions are made on pages 9 and 10 of the Central District Committee's report.

POWDERS.

Whether in bulk or in packages, no special difficulties present themselves. The square-root principle, and the principles laid down in the U. S. Pharmacopeia, p. 463, will be quite applicable.

SEMI-SOLIDS.

Substances falling into this group are probably the most difficult to deal with in connection with the question of sampling. If they are sufficiently soft, they may, of course, be mixed with a stick or paddle until uniform, but if they are too thick and sticky for such mixing, they present a most perplexing problem. Asafoetida is an extreme example. Suggestions are given by Schultz in his report of July 19, 1917, page 7, b, and in Central District Report, May 26, 1919, page 39. But there is a doubt that these are at all sufficient, and it is suggested that this group be divided into very small groups or even individual items and details evolved by the committee to assist in properly sampling this group.

PILLS—TABLETS.

In connection with the sampling of these classes, and particularly of tablets, the following suggestions were made by the writer on January 20th, 1925, under I. S. Nos. 19555-56-V:

1.—*In case of units containing 1000 or more tablets.* In such instances there is usually secured only one unit.

Open and cautiously mix the entire contents, being careful not to mutilate the tablets unnecessarily, and divide into two parts. One of these should be of such size as to represent a liberal subsample for the original analyst. Usually one-third will be satisfactory. Return the other portion to the original bottle and seal for use by check analyst, or in any manner which may subsequently appear necessary Accurately weigh the analyst's subdivision and count the individual tablets. This will give the exact average weight per tablet Grind and mix the entire subsample and weigh out portions for analysis.

2.--*In case of units containing 100–500 tablets.*

If more than one unit has been collected, weigh the entire contents of one unit, count the tablets, grind to a fine powder, and weigh out portions of the powder for analysis.

If only one unit is available, but there is sufficient material to warrant subdividing, proceed as directed under 1 If it is not considered that there is sufficient material to warrant subdividing, proceed as directed above.

3.—*In case of small units, such as tubes of hypodermic tablets.*

In such instances a number of units are usually available. Choose such number of units as will constitute a satisfactory analyst's sample. Reserve the rest of the tubes for check analysis or such purpose as may subsequently appear necessary.

Weigh all the tablets in all the tubes of the analyst's subdivision, and count the tablets. Grind, and weigh out portions for analysis.

4.—*Tablets containing small dosages, as for example, 1/100 grain, of active ingredient.*

In such instances, the number of tablets required for a single determination may be so large as to render grinding unnecessary. An entire bottleful, or one-half a bottleful, may be required. Under such circumstances, count the tablets and use them directly for the determination. The weight may well be recorded also

AMPOULES.

It would seem that ampoules should be considered in a class by themselves. Each unit is in effect a treatment, and each should be perfect in every way. It would hardly seem necessary to secure, in each case, as many units as would be desirable in the case of other preparations. It would rather seem that such number of units as would be needed for analysis, check analysis, and any other emergency, might be sufficient.

Since practically no information is given in the literature relative to proper sampling, this subject should be given careful consideration by this committee when work on the drafting of final methods is undertaken.

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Paper, Sampling Crude Drugs, *J. Am. Pharm. Assoc.*, 1924, **13**: 212.
Letter, to writer from F. O. Taylor, Chief Chemist, Parke-Davis & Co., 12/2/25.
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U. S. P. Revision Circular 300, p. 1700—Methods for Sampling and Analysis of Vegetable Drugs.
U. S. P. Revision Circular 400, p. 2269—Comments on Botany and Pharmacognosy.
Paper, Crude Drug Sampling, by L. J. Schwartz, presented at meeting of American Drug Manufacturers Association, New York, Spring of 1925.
Letter, to writer from G. J. Morton, Chief, San Francisco Station.

REPORT OF COMMITTEE TO CONSIDER THE ADVISABILITY OF STUDYING METHODS FOR THE ANALYSIS OF PAINT.

No formal report was prepared. The chairman of the committee made the following remarks:

W. F. Hand: I have no report to make as the association did not have an opportunity to vote on the report of the committee last year. I was unable to be here but I sent the report to Dr. Skinner, and through some delay it was not delivered to him in time. However, the report and recommendations were published in *The Journal* with an explanatory note[1].

Your committee has done considerable work collecting information on paint, and it seems very evident that we have a field of usefulness here. There is no harmony of view, however, with reference to law and regulation, and it seems to us that it will be difficult to stimulate any enthusiasm until more paint work is done by the various State departments. There are about 20 States having paint laws, and these States are represented here, but it is true that the laws are not being very fully enforced, and that there are few published data that refer to the work that has been done. It will be difficult to do any systematic work until we obtain better information with reference to what the law and regulations should be with regard to the inspection of these products, but we believe that finally this matter will develop until it will require a separate section of the association.

However, the committee wishes to repeat the recommendations made last year, which are as follows:

(1) The appointment of a permanent committee of five members interested in paint and oil control work.

(2) That the Paint Committee be instructed to begin cooperative work with the State departments engaged in paint law enforcement with the view to developing a suitable law or laws; to bringing about the uniformity of laws; and to developing and unifying regulations, definitions, and standards where necessary.

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[1] *This Journal*, 1926, **9**: 107.

(3) That the committee be asked to cooperate in the development of methods of paint examination and analysis, to seek cooperation of paint chemists within this association's membership and elsewhere; and to cooperate with the work of the Committee on Protective Coatings of the American Society for Testing Materials with the view to developing suitable procedures for the evaluation of paints, oils, and paint products.

Approved.

REPORT OF COMMITTEE ON BIBLIOGRAPHY.

It may be remembered that last year the Committee on Bibliography was continued as a permanent committee. The report for this year is one of progress.

The committee was very fortunate in securing the following assistants: B. B. Ross to prepare the review on Potash, R. N. Brackett for the work on Phosphoric Acid, H. B. McDonnell for the work on Nitrogen, F. D. Fuller for the work on Cattle Feeds, W. H. MacIntire for the work on Soils, and C. C. McDonnell for the work on Insecticides.

The plan that was being developed by Mr. Doolittle and the chairman was to have a review and bibliography for each of the chapters in *Methods of Analysis*. It had proceeded to a point where a number of additional people had been selected, and certain correspondence had been had with them. It was interrupted by Mr. Doolittle's untimely death, as the chairman was expecting him to work out the outline for the men on the remaining chapters. The very last letter received from him was about this matter of the bibliography. The work will go forward, it is expected, and will follow out the plan presented last year. It was not the thought, however, that the reports of the reviewers would be presented before next year. It is planned to have all these reviews in shape by the time of publication of the next edition of *Methods of Analysis*.

W. W. SKINNER,
G. S. FRAPS,
F. P. VEITCH,
H. D. HASKINS,
W. W. RANDALL.

Committee on Bibliography.

Approved.

REPORT OF COMMITTEE ON CONSTITUTION AND BY-LAWS.

B. B. Ross, the chairman of this committee, was obliged to leave the association before presenting any report. The following report was given by a member of the committee:

F. P. Veitch: As Dr. Ross had to leave, he requested me to present the report. When the members of the committee got together to consider the suggested changes in the by-laws, it seemed there were so many places where some change appeared, on the surface, to be necessary, and so many different interests to be considered, that it was felt that nothing final could be done at this time. Instead, the committee offers these recommendations:

(1) That the membership of the committee be increased to five, with a view to securing representation from the chief groups of workers interested in the activities of the association, and

(2) That the report of this committee as to proposed amendments, or modifications of provisions of the constitution and by-laws be published in the last number of *The Journal* of this association issued in advance of the next meeting of the association.

In this way the matter may receive the careful consideration of each individual member, and the committee will not be obliged to make any decisions without time for consideration. It is hoped that everyone will give this matter thorough consideration and that the association may take the proper action when this report comes up next year.

Approved.

REPORT OF AUDITING COMMITTEE.

The Auditing Committee has examined the accounts of R. W. Balcom, Chairman of the Board of Editors, covering the period from October 15, 1925, to October 1, 1926, and found the same to be correct as reported.

The committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from October 15, 1925, to October 1, 1926, and found the same to be correct as reported.

H. B. McDonnell,
J. B. Weems.
Auditing Committee.

Approved.

REPORT OF NOMINATING COMMITTEE.

The committee desires to present the following names:

President: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

Vice-President: Oswald Schreiner, Bureau of Plant Industry, Washington, D. C.

Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry, Washington, D. C.

Executive Committee: E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.; L. D. Haigh, Missouri Agricultural Experiment Station, Columbia, Mo.

JULIUS HORTVET, F. C. BLANCK.
A. J. PATTEN,
Nominating Committee.

It was moved, seconded, and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

W. H. MacIntire: MR. PRESIDENT, AND MEMBERS OF THE ASSOCIATION: One thing I noticed in our retiring president's announcement. Usually he is exceedingly careful as to terminology, but he announced us as "candidates" rather than as "nominees". I would, therefore, like to say that we were not candidates. He also failed to ask us whether or not we would accept; he seemed to be very sure that we would.

Now, I have just enjoyed going through the proceedings of this association for a number of years, because of an assignment in working up a certain bibliography. I find you have had forty-two presidents and forty-two acceptances. I think it is rather dangerous to create a precedent, and I do not feel that I should do so by declining this honor. I realize, however, my own limitations, and when I look over the past, I feel that the honor accorded me isn't fully merited. I thank you for your good fellowship. I think your good fellowship is more generous than your judgment is dependable. Nevertheless, I shall try to serve you. I should like, Mr. President, to have you continue to preside during the remainder of the meeting.

REPORT OF COMMITTEE ON RESOLUTIONS.

MR. PRESIDENT AND MEMBERS OF THE ASSOCIATION:

The committee records with deepest sorrow the death of one of the most honored members of the association, Roscoe Edward Doolittle. He died at his home in Evanston, Ill., April 25, 1926, at the age of 51.

Words are inadequate to express the sense of loss of this co-worker, who for 25 years has been an active member of this organization, serving with distinction in every position of honor within its gift,—as referee on various subjects, chairman of its most important committees, editor of its publications and, in 1924, as its president.

Mr. Doolittle was born in Michigan and was graduated from the Agricultural College of that State. After special preparation in food analysis at Ann Arbor, he served as analyst for the Michigan Dairy and Food Commission for eight years, entering the Bureau of Chemistry in 1904 in charge of the New York laboratory organized for the inspection of imported food products. His judgment and experience proved of greatest value when the food and drugs act took effect. He remained in charge of the New York laboratory until 1911, when he became a member of the Board of Food and Drug Inspection and, in 1912, the acting chief of the Bureau of Chemistry. After the establishment of inspection districts he was successively in charge of the Eastern and Central Inspection Districts.

In all of those varied positions he exercised rare ability, judgment, and tact, and his personality was such as to endear him to his associates.

He was a member of the American Chemical Society, the American Association for the Advancement of Science, the American Pharmaceutical Society, the American Public Health Association, and the Association of Official Agricultural Chemists. He was a tireless and conscientious worker with keen judgment and high ideals.

He gave freely of his thought and energy to this association and the *Book of Methods* will remain a lasting monument to his memory.

The committee recommends the adoption of the following resolutions:

Resolved, That this association desires herewith to record its high estimate of the distinguished services rendered by Roscoe E. Doolittle, not only to this organization but for the promotion of the public welfare, and to express its profound sorrow at his death.

Resolved, That the foregoing resolution be printed in the proceedings of this association and that a copy, including the preamble, be sent to the family of our deceased friend and associate.

The committee also recommends the adoption of the following resolutions:

Resolved, That this association extend its felicitations to its honorary president, Harvey W. Wiley, on this occasion of the 20th anniversary of the adoption of the Food and Drugs Act, to proffer its congratulations upon his record for long and distinguished services to this association and as a public benefactor, and to express its thanks for the stimulating influence of his presence at this meeting.

Resolved, That this association desires to express to its president, W. W. Randall, its sincere appreciation of the gracious and efficient manner with which he has conducted this anniversary meeting.

Resolved, That this association tender its sincere thanks and appreciation to W. W. Skinner, W. S. Frisbie, Miss Marian E. Lapp, and her charming assistants, whose joint labors have so largely contributed to the success of this meeting.

Resolved, That this association is greatly indebted to the chairman of the Board of Editors, R. W. Balcom, and to his associates for the progressive and efficient manner in which *The Journal* of this association has been conducted, and desires to congratulate them upon its success.

Resolved, That the thanks of this association be and are hereby given to the management of the New Willard Hotel for the use of its halls as a place of meeting and for the many courtesies extended to its members.

A. S. MITCHELL, JAMES W. KELLOGG.
F. D. FULLER,
Committee on Resolutions.

Approved.

The Thursday morning session continued until 1 p. m., when all the reports had been presented. No afternoon session was held. At 2 p. m. members and visitors took a trip to Arlington and Mount Vernon.

The proceedings for Monday, Tuesday, and Wednesday will be published in Nos. 2, 3, and 4 of Volume X.

CONTRIBUTED PAPERS.

A RAPID METHOD FOR THE DETERMINATION OF STARCH.

By O. S. Rask (Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.).

PRELIMINARY EXPERIMENTS.

A little preliminary work showed that cold and relatively concentrated hydrochloric acid is capable of dispersing starch into a clear or slightly opalescent but filterable solution, out of which the starch can be precipitated or coagulated quantitatively by approximately two volumes of alcohol. These reactions have been used as the basis for a rapid and apparently accurate method for estimating starch directly instead of indirectly in the form of reducing sugars.

Starch seems to undergo no deep seated changes in this process of acid dispersion, followed by alcoholic coagulation. It still gives the characteristic blue color with iodine, and it has no reducing action on Fehling's solution. The only noticeable changes in the starch are that the original morphology or identity of the grains has been destroyed and that the starch itself has been rendered water-soluble. With cold water it forms an opalescent solution. If this opalescent solution is centrifuged a flocculate settles in the bottom of the tube, leaving a perfectly water-clear supernatant solution which continues to give an intense or deep blue color with iodine, and which has no reducing action on Fehling's solution. Apparently the flocculate is alpha amylose, which constitutes about 15 per cent of the starch grain and occurs mainly in the outside layer. The supernatant water-clear liquid can be nothing else than a solution of beta amylose, which represents the remaining 85 per cent and interior of the starch grain[1].

Very little consideration seems to have been given to the action of cold and relatively concentrated hydrochloric acid on starch, although Wiley (1914)[2] mentions a method for estimating starch by its optical activity when dissolved in concentrated hydrochloric acid. Such a solution of starch is water clear. When starch is treated with slightly diluted acids, opalescent solutions result. This opalescence grades into turbidity as more diluted acids are used.

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[1] Another interesting property of this coagulated starch is that it will form a so-called "artificial milk," or permanent suspension, when treated in the same manner that emulsions or suspensions of hydrophobic colloids are stabilized by hydrophylic colloids, which are then designated as protective An illustration is the familiar stabilization of oil in water emulsions by gums like gum acacia In that case the oil is rubbed in a mortar with the gum and a small quantity of water until a distinctive and characteristic cracking sound is heard. The resulting product when diluted and stirred with water forms a permanent emulsion or so-called "artificial milk" due to the protective action of the hydrophylic gum on the hydrophobic oil. When the above acid-dispersed and subsequently alcoholically coagulated starch is rubbed in a mortar with approximately its own weight of water a similar cracking sound is heard within a few moments. If the starch is then stirred up in water an "artificial milk" results. This starch milk is such a permanent and stable dispersion that prolonged centrifuging has no apparent effect on it. A plausible explanation of its structure is that the hydrophobic alpha amylose has been permanently dispersed and suspended by the protective action of the hydrophylic beta amylose. Accordingly in this starch milk the positions of the alpha and beta amyloses with respect to one another are the reverse of what they are in nature. In the original and natural starch grains the alpha amylose coats the beta amylose, but in the above milk the beta amylose coats the alpha amylose

[2] Principles and Practice of Agricultural Analysis, 2nd ed., Vol. 3, p. 287.

In all these solutions starch undergoes a gradual hydrolysis, which may be demonstrated by simple and well-known tests. The rate seems to vary rather directly with the concentration of the acid, and obviously also with the temperature. At room temperature (20°-22°C.) a solution of starch in concentrated hydrochloric acid will react positively with iodine for a period of 35–50 minutes, after which the test is negative, indicating a complete hydrolysis of the starch into more simple carbohydrates. In three volumes of concentrated hydrochloric acid and one of water the blue starch iodide reaction is positive for 3 to 4 hours; in two volumes of concentrated hydrochloric acid and one of water the reaction is positive for 5 to 6 hours; and in equal volumes of concentrated hydrochloric acid and water the reaction is positive for 24 hours.

Under these conditions the negative iodine reaction marks the end rather than the beginning of a hydrolyzing process, which must be avoided in order to coagulate the starch quantitatively by means of alcohol. It was necessary, therefore, to ascertain conditions of acid dispersion which would not involve any hydrolysis of starch sufficient to prevent its complete coagulation by alcohol. Obviously in such acid dispersions the onset of starch hydrolysis is a matter of time, temperature, and acid concentration. The higher the temperature or acid concentration the shorter will be the time within which hydrolysis begins to set in. No attempt was made to determine any large number of combinations of these three factors, namely, time, temperature, and acid concentration, under which starch could be recovered completely from a hydrochloric acid solution by means of alcoholic coagulation. Consideration was confined to those combinations which showed promise of making the simplest and the most rapid technique for estimating starch with reasonable accuracy. For this reason time and acid concentration were standardized on room temperature (20°–25°C.), which preliminary experiments had shown to be satisfactory for this purpose.

Acid concentration was first considered. Obviously the optimal concentration would be the lowest concentration capable of dispersing starch grains into filterable solutions at room temperatures, since this concentration would have the slowest hydrolizing action on starch. Furthermore, this lowest possible concentration would also have the least action on celluloses and hemicelluloses. In order to determine this concentration the following experiments were performed: About 0.1 gram of corn starch was placed in each of a series of test tubes. Just enough water (3 or 4 drops) was added to each tube to moisten the starch, for in the dry condition starch cakes or lumps when brought into contact with acid sufficiently concentrated to disperse it. To the first tube was added 15 ml. of a solution of hydrochloric acid containing 22 grams of hydrogen chloride per 100 ml. solution; to the second tube was added 15 ml. of a solution of hydrochloric acid containing 20 grams of hydrogen chloride per 100 ml. solution; to the third tube was added 15 ml. of a solution of hydrochloric acid containing 19 grams of hydrogen chloride per 100 ml. solution; and to the fourth tube was added 15 ml. of a solution of hydrochloric acid containing 18 grams of hydrogen chloride per 100 ml. solution. In the case of each tube the contents were stirred immediately after the acid was added. The starch in the first and second tubes dispersed into clear though slightly opalescent solutions almost as soon as the stirring was started. The starch in the third tube dispersed with the same completeness after a few moments' stirring. The starch in the fourth tube reacted rather indefinitely; even after prolonged stirring some of it appeared unaffected, although a large part of it had undoubtedly been dispersed. These tests were next repeated on two different wheat starches and then on a potato starch. The reactions of the wheat starches were essentially the same as those of the corn starch. However, the potato starch dispersed in more dilute solutions. A hydrochloric acid solution containing 18 grams of hydrogen per 100 ml. solution sufficed to produce an almost water-clear dispersion of this starch, and a hydrochloric acid solution containing 16 grams of hydrogen chloride per 100 ml. solution produced of this particular potato starch a dispersion which was clearer, or at least less opalescent, than the dispersions of the other starches in solutions containing 18 grams of hydrogen chloride per 100 ml.

These rather simple tests indicated that the ability of cold hydrochloric acid to disperse starch depends upon a rather critical concentration. In case of the starches examined, this concentration is approximately 19 grams of hydrogen chloride per 100 ml. solution when applied to starch previously moistened with a volume of water, which represents about 1 per cent of the volume of the acid added. It may be mentioned at this point that in these preceding experiments care was taken to add a volume of acid of which the starch-moistening water represented about 1 per cent. This was done in anticipation of a similar dilution of the dispersing acid by the residual wash water contained in the analytical sample to which the dispersing acid would be applied. In other words, the concentration of the dispersing acid was standardized on the basis of a subsequent 1 per cent dilution by residual wash water contained in the analytical sample to which the dispersing acid would be applied.

In order to ascertain whether dispersions of starches in acids containing 20–21 grams of hydrochloric acid per 100 ml. solution were filterable, particularly whether a Gooch asbestos filter would retain or filter out any of the apparently dispersed starch, the following test was made in duplicate: A one gram portion of wheat starch was moistened with 1 ml. of water and then dispersed in 99 ml. of hydrochloric acid containing 20 grams of hydrogen chloride per 100 ml. The dispersion was then allowed to stand for about 10 minutes, after which it was filtered through a previously oven-dried and weighed Gooch asbestos filter. This 10 minute interval was allowed since it was apparent that in the proposed method for estimating starch at least 10 minutes would elapse from the time the acid comes in contact with the starch until filtration is started. The Gooch filter, after the starch dispersion had passed through it, was washed twice with fresh portions of the same acid solution, then twice with water, and finally once with alcohol and once with ether. It was then dried in the drying oven, cooled, and weighed. The resulting data are as follows:

	No. 1 *grams*	No 2 *grams*
Weight of crucible after filtration	13.9913	13 9904
Weight of crucible before filtration	13 9873	13 9868
Residue	.0040	.0036

The residues in the crucibles, it will be noticed, represent 0.4 per cent, or less, of the original starch. However, this residue failed to give a blue color with iodine so that it probably consisted of non-starch material present in the sample. This non-starch material may have been the same as refinery mud which has been described by Nelson and Taylor[1]. In that case it may be concluded that starches dispersed in the above manner are filterable. In all subsequent work there was no evidence that acids of the above concentrations failed to disperse previously moistened starches into filterable solutions under the above conditions, although acid solutions containing as low as 20 grams of hydrogen chloride per 100 ml. solution produce starch dispersions which filter more slowly than do dispersions in acids containing 21 grams of hydrochloric acid per 100 ml. of solution. The former dispersions seem to be somewhat more viscous than the latter. For this reason it was decided to specify arbitrarily 20.5–21.0 grams of hydrogen chloride per 100 ml. as the limits of variability in the hydrochloric acid to be used for dispersing starch in the proposed technique. In order to allow an extra quota of hydrogen chloride to satisfy any acid-binding capacity of proteins in the sample, it is probably advisable to use hydrochloric acid solutions whose concentrations border on high limit, that is, 21 grams of hydrogen chloride per 100 ml. of solution.

It is interesting to note, at this point, that hydrochloric acids of the above concentrations have no significant action on celluloses and hemicelluloses at ordinary room temperatures. Evidence to that effect can be produced conveniently by the use of starch-free wheat bran prepared from ordinary commercial bran by washing out the greater part of the starch and removing the rest by heat gelatinization and diastatic action followed by water extraction. Such bran, although it contains 10–12 per cent of crude fiber and 20–30 per cent of pentosans, yields with the above hydrochloric acid solutions an extract from which nothing precipitates when it is poured into two volumes of alcohol. This extract also gives a very doubtful or negative Molisch test.

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[1] *J. Am. Chem. Soc.*, 1920, **42**: 1726.

The next point to be investigated was the time limit within which starch could remain dispersed in hydrochloric acid under the above conditions without undergoing hydrolysis sufficient to prevent its complete coagulation by alcohol. One-half gram portions of wheat starch previously moistened with 0.5 ml. of water were dispersed in 50 ml. portions of hydrochloric acid solutions containing 21 grams of hydrogen chloride per 100 ml. of solution. These solutions were maintained at different temperatures ranging from 20°–25°C. and for varying time periods up to one hour. The solutions were then poured into a little more than two volumes of alcohol. The resulting mixture was stirred until flocculation of the starch seemed complete. After the flocculate had settled out a portion of the supernatant solution was decanted through a Gooch filter and tested for carbohydrates by the Molisch reagent. It was impossible to distinguish sharply between a negative and a positive Molisch reaction. However, it appeared that filtrates from those coagulated starches that had previously remained dispersed in the acid for 45 minutes at 25°C. gave a faintly positive reaction for carbohydrates. On the other hand, a distinctly negative reaction was given by the filtrates from starches that had been coagulated after being dispersed in the acid either for a shorter period or at a lower temperature. No differences could be detected in the reactions of wheat, corn, and potato starches to these tests. Therefore, in all probability, starches dispersed in hydrochloric acid solutions containing 21 grams of hydrogen chloride per 100 ml. solution at 25°C. for a period less than 45 minutes or at a temperature lower than 25°C. for 45 minutes may be coagulated quantitatively by means of alcohol.

In developing this method other problems and considerations of relatively minor importance arose. They are indicated and explained adequately in the following text of the method and the appended notes.

THE METHOD.

Transfer 1–4 grams (1) of the material, which must be finely ground, to a funnel fitted with a 7–9 cm. filter paper, and extract successively with washed ethyl ether, 10 per cent alcohol (2), and water, using the solvents in the order named and filling the filter four or five times with each solvent. After the wash water has drained out, transfer the material, together with the filter paper (3), to a 50 ml. beaker. Add 10 or 11 drops of cold (4) hydrochloric acid containing 20.5–21.0 grams of hydrogen chloride per 100 ml. solution. Then tamp the material by means of a heavy stirring rod with a flattened end 15 mm. in diameter. Continue this tamping until the contents of the beaker have been converted into a paste entirely free from lumps. Then add 20–25 ml. of the same cold hydrochloric acid and stir until a smooth and uniform suspension results. Transfer this suspension to a 100 ml. volumetric flask that has previously been recalibrated so as to allow for the volume occupied by the filter paper. Rinse the beaker with fresh portions of the same hydrochloric acid solution and add the rinsings to the 100 ml. flask; finally fill the flask to the mark with the same acid solution and mix the contents thoroughly by shaking. Filter the contents with the aid of suction through a Gooch crucible fitted with a dry asbestos mat, and two-thirds full of dry and fluffy asbestos, and receive the filtrate in a small dry suction flask (6). Transfer by means of a calibrated pipet, 50 ml. of the filtrate into a 200 ml. beaker containing 110–115 ml. of 96 per cent alcohol (7). (This step must be completed within 35 minutes of the initial contact of the acid with the starch, in order to avoid any appreciable hydrolysis of starch (8).) Immediately after the 50 ml. pipet has drained completely, but not until then, stir the resulting mixture continuously for about 1 minute, or until the precipitate has flocculated rather completely. Set the beaker aside until the precipitate has settled, then decant (9) the supernatant liquid through a Gooch crucible previously fitted with an asbestos mat and brought to a constant weight. In this decantation hold the precipitate back in the beaker as completely as possible in order to avoid a slow filtration (10), and then wash it (in the beaker) with two or three successive 15 ml. portions of 70 per cent (by volume) alcohol, then once with 96 per cent alcohol (11), decanting each portion through the Gooch crucible. In this washing tamp and disintegrate the flocculated starch by means of a heavy stirring rod flattened on the end in order to remove occluded impurities, particularly hydrochloric acid, which, if not washed out, will cause a charring of the starch in the subsequent drying at 100° or 130°C. After washing with the 96 per cent alcohol, transfer the precipitate to the Gooch by means of 96 per cent alcohol from the wash bottle, and finally wash the precipitate once with 20-25 ml. of ethyl ether, preferably anhydrous. Dry the crucible with contents for one hour at 130°C., at 100°C. in vacuo, or at 105°C. atmospheric pressure to a constant weight Cool 20-30 minutes in a desiccator provided with phosphoric anhydride or freshly ignited calcium oxide, and weigh as starch. Because of the great hygroscopicity of the starch the crucible must be covered when weighed, but it is not necessary to provide each crucible with a separate cover; the same cover can be kept in the balance case and used for all weighings.

Until familiarity has been acquired with this technique, it will be advisable to test the acid-alcoholic filtrate for starch by means of iodine, and for carbohydrates in general by Molisch's reagent. Both tests should be negative. Qualitative tests for proteins, such as the Biuret test, Millon's reaction, and the Ninhydrin test, may be applied to the precipitated starch after it has been weighed. These tests should also be negative.

NOTES.

1. It is desirable to start with a sample that represents 0.5-1.0 gram of starch. The quantity of starch finally weighed will then vary from 0.25–0.5 gram.

2. If the material is wheat flour, it may be advantageous to destroy some of its gluten-forming properties by two or three extractions with 70 per cent (by volume) alcohol, after extracting with 10 per cent alcohol and before extracting with water, or 70 per cent alcohol may be used in place of the 10 per cent alcohol, thereby omitting the 10 per cent alcohol entirely. Be certain that the water has removed all the alcohol before the material is treated with hydrochloric acid.

3. The bulk introduced by the filter paper may be reduced somewhat by tearing away the folded portion that has not been in contact with the material.

4. The temperature of the acid must not exceed 22°C. during any part of the determination. Lower temperatures are not objectionable. A convenient way of keeping the temperature of the acid below the required limit is to keep the bottle in the ice box. It will then have a temperature around 10°C. From such a temperature the acid will ordinarily not rise above 20° or 22°C. within 35 minutes, even on a warm summer day.

5. These values denote concentrations of gaseous hydrogen chloride. They should be checked by titration and must not exceed the specified limits; 0.5 ml. of this acid should be equivalent to 28 1–28 8 ml. of 0.1 *N* acid.

6. The loose and fluffy asbestos prevents a clogging of the mat by proteins and other gummy substances.

7. If the alcohol is added to the acid starch solution a large portion of the precipitated starch will stick tenaciously to the walls of the beaker. This difficulty is avoided entirely by adding the acid starch solution to the alcohol.

8. Cold hydrochloric acid containing 20–21 grams of hydrogen chloride per 100 ml. hydrolyzes starch slowly, but the extent of hydrolysis during the first 30–45 minutes of contact does not seem to be appreciable, as indicated by tests for sugars in the acid-alcohol filtrate.

9. The coagulated starch will occasionally rise to the top of the acid-alcohol solution if allowed to stand for any length of time. Decantation and, consequently, filtration also will then be very slow and difficult. In order to avoid such a situation the decantation should be started immediately after the coagulated starch has settled to the bottom of the beaker.

10. Precipitated starch in contact with 70 per cent alcohol is mucilaginous or gummy in its consistency and in this form will clog a Gooch filter, but the same starch when treated with 95 per cent alcohol becomes granular. In this form it has no appreciable retarding effect on filtration.

11. In order to get a more complete effect of the dehydrating action of strong alcohol, the starch is washed once in the beaker with 96 per cent alcohol before being transferred to the Gooch crucible.

APPLICATION TO PURIFIED STARCHES.

This method was first applied to raw or ungelatinized starches of wheat, corn, and Irish potato. These starches had been prepared, purified, and desiccated by methods which have been described by Hermano and Rask (1926)[1]. In applying the method to these starches the preliminary washings or extractions with ether, dilute alcohol, and water were omitted, as these had been included in the final purification of the starches. One gram samples, therefore, were transferred directly to the 50 ml. beaker, after which sufficient water (1.0–1.5 ml.) was stirred into the sample to moisten it or to make a stiff raw paste. This paste was then stirred into 25–30 ml. of the cold hydrochloric acid containing 20.5–21.0 grams of hydrogen chloride per 100 ml. of solution, and the resulting dispersion was transferred to the 100 ml. volumetric flask, the transfer being made quantitatively by means of additional portions of the hydrochloric acid solution. From this point the method, as given previously, was followed, except for the following additional procedure: Weighed Gooch crucibles were used in the filtration of the acid-starch dispersion as well as in the subsequent filtration of the alcoholic coagulated starch.

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[1] *Cereal Chem.*, 1926, 3: 361.

After the 100 ml. acid-starch dispersions had passed through these crucibles they were washed with a fresh portion of the same hydrochloric acid solution, then with water, 95 per cent alcohol, and absolute ether, and finally dried in the oven, cooled, and weighed. This additional step was introduced in order to ascertain whether the acid-starch dispersion was completely filterable or whether any of the starch would be filtered out by an asbestos mat of this type. No other changes were made in the method in applying it to these starches, which were afterward analyzed for their non-starch constituents by official methods of the Association of Official Agricultural Chemists[1], except in the case of combined fat, which was assumed to be 0.5 per cent. The results are given in Table 1.

TABLE 1.

Results on purified starches.

	WHEAT STARCH *per cent*	POTATO STARCH *per cent*	CORN STARCH *per cent*
Starch	93.18 93.56	93.26 93.70	96.00 96.14
Average	93.37	93.48	96.07
Moisture	4.62	4.19	2.35
Protein	0.23	0.34	0.40
Ash	0.06	.	.
Combined fat (assumed)	0.5	0.5	0.5
Total	98.78	98.51	99.32

Perhaps the most conspicuous values in Table 1 are the totals. These show recoveries which are probably as complete as can be expected from materials of this kind. The slight discrepancies between the totals and 100 per cent may be due in part to possible errors in determining moisture. It is possible that the official method of drying at 130°C. for one hour does not expel the last traces of moisture from starch. These discrepancies may also be due in part to combined fat, which has been assumed to be 0.5 per cent. This value was assumed because Nelson and Taylor (1920) showed that starches contain at least this amount of fat that cannot be removed by ordinary ether extractions. However, the data of these investigators do not show that the amount of such fat is not greater, as may have been the case in the above starches.

There are other and more direct evidences of the efficiency of this method in recovering starch than those contained in Table 1. Some of these evidences were supplied by the weighed Gooch asbestos filters through which the acid-starch dispersions were filtered. These filters gained 0.0020 to 0.0040 gram as a result of this step. These weights represent 0.2 to 0.4 per cent of the original samples. These percentage values are insignificant since they no doubt come within the limits of probable accuracy of the method. More significant is the fact that this material in the Gooch asbestos mat and represented by these gains in weight failed to give a blue color with iodine. It was, therefore, not starch, but more probably "refinery mud" which has been discussed by Nelson and Taylor. Accordingly, no starch can be regarded as lost or removed in the filtration of the acid dispersion.

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[1] *Methods of Analysis, A. O. A. C.*, 1925.

Other evidences of a complete recovery of starch by this technique were the uniformly negative tests on the acid-alcoholic filtrates for starch by iodine, for reducing sugars by Fehling's solution, and for carbohydrates in general by the Molisch reagent. These negative reactions preclude a partial hydrolysis of starch into products not coagulable by alcohol, an incomplete coagulation of starch itself by alcohol, a partial solubility of the coagulated starch in the washing solutions, *viz.*, 70 per cent and 95 per cent alcohol and absolute ether, and any inability of the asbestos mat to retain the coagulated starch. As no other possibilities of starch losses are conceivable, the recovery of starch by this technique may be regarded as complete and quantitative.

SPECIFICITY OF THIS METHOD FOR STARCH.

Three different procedures were used for determining the extent to which this method is specific for starch. The first consisted of applying the method to biological products that are known to be free from starch. In this case specificity is indicated by the absence of any coagulum such as is formed by starch. The second involved application of the method to a synthetic mixture containing a known quantity of starch. Specificity is then indicated by a yield of starch equal to the quantity of starch known to be present in the mixture. The third procedure consisted of testing the purity of coagulated starches obtained by means of the method from products of relatively complex composition. In that case specificity is indicated by the extent to which non-starch substances are absent.

The starch-free substances to which the method was applied for the purpose of ascertaining its specificity for starch according to the first procedure were starch-free bran, of which mention has already been made, cottonseed meal, and a mixture of approximately equal parts of cottonseed meal and powdered water-soluble egg albumin. None of these products gave the slightest coagulum when subjected to the proposed method for estimating starch. Both the cottonseed meal by itself, and the mixture of it and the water-soluble egg albumin failed to give any coagulum even when their preliminary extractions with ether, dilute alcohol, and water were omitted. Obviously these experiments demonstrate that the method possesses a very high specificity for starch. They show that none of the numerous and complex constituents of the above products are coagulated by the method.

Even though the non-starch constituents are not coagulated directly by this method it seemed possible that some non-starch constituents might be occluded or adsorbed to an appreciable extent by starch in the process of its coagulation by alcohol. In order to test this possibility the second procedure of ascertaining specificity was employed, in which the following experiment was carried out in duplicate. One gram of the corn starch listed in Table 1 was transferred to a funnel fitted with a 7 cm. filter paper. Over this 1 gram of starch was placed 0.6 gram of the cottonseed meal mentioned previously. The contents of the funnel were then subjected to the method, including the preliminary extractions with ether, dilute alcohol, and water. The results were 96.02 per cent and 96.18 per cent of starch. By reference to Table 1, it will be noticed that these percentages are practically identical with those given by the starch itself in the absence of cottonseed meal. It seems very probable, therefore, that the adsorbing or occluding capacities of starch coagulated by this method are negligible, at least for all ordinary analytical purposes.

The third procedure of testing the specificity of the method for starch consisted of determining the nitrogen and ash contents of coagulated starches which had accumulated as a result of a rather general use of the method for determining starch in whole wheat, wheat germ, and wheat flour in the laboratory. Nitrogen was determined in duplicates, each consisting of 2 grams of the coagulated starch. The official Kjeldahl method, in which mercuric oxide is the catalyst, was used. The results were 0.053 per cent and 0.040 per cent of nitrogen. These are equivalent to less than 0.3 per cent of protein, a quantity which can be regarded as insignificant, because it does not affect analytical results beyond the limits of accuracy of the method.

Ash was determined by a direct incineration of the coagulated starch in the original Gooch crucibles, into which the starch had been filtered. Each of these crucibles had been ignited previously in order that ash might be determined in this manner. In no instance was a weighable ash obtained.

These few and simple tests warrant the conclusion that celluloses, hemicelluloses, proteins, and mineral matter are not likely to be present in starch as coagulated by this method. It must be realized, however, that there are such wide natural variations among such non-starch substances that no final or sweeping conclusions can be drawn regarding their general non-interference without further tests on a larger variety or selection of material.

Possibilities of contamination of the coagulated starch by fats are very remote or almost inconceivable from the general nature of the method.

In the initial treatment of the original material fats are almost completely removed by ether extraction. Any remaining fat is probably filtered out in the first filtration along with celluloses, hemicelluloses, and some of the proteins. Any of the fat which may find its way into the filtrate of the first filtration will probably do likewise in the second filtration, in which 95 per cent alcohol and absolute ether are the final washing media. Therefore, the quantity of fat remaining in the alcohol and ether-washed coagulated starch will, in all probability, be insignificant, at least for all ordinary analytical purposes.

Possibilities of contamination of the coagulated starch by the simpler carbohydrates are equally remote. Any of these that may be present in the original material are washed out very largely, if not entirely, in the preliminary extractions with dilute alcohol and water. Furthermore, these carbohydrates are soluble in the 68–70 per cent alcohol in which the starch is coagulated and by which it is subsequently washed. However, this cannot be said of certain polysaccharides—like pectins and dextrin—which are insoluble in 65–70 per cent alcohol. These polysaccharides, if present in small quantities, would probably be removed with sufficient completeness in the preliminary extractions of the material with dilute alcohol and water, but large quantities would no doubt give trouble. Accordingly, the method in its present form cannot be regarded as applicable to exceptional products like linseed meal that contain more than a trace of polysaccharides, such as dextrins and pectins.

Another phase of specificity is the completeness with which the method recovers starch. Evidence on this phase was obtained in a rather incidental manner in preparing the starch-free bran which has already been mentioned. An attempt was first made to prepare such bran from ordinary commercial bran by washing out the greater part of the starch and removing the rest by diastatic action according to the official diastase method for determining starch[1]. That is, the washed bran was suspended in water, which was then heated up to about 95°C. on the steam bath, then cooled to 55°C., and treated with malt diastase at this temperature for 30 minutes. The suspension was then heated on the steam bath a second time to 95°C., cooled to 55°C., and treated at this temperature with a second and fresh portion of malt diastase until the digesting mixture failed to give a positive test for starch by iodine. The bran was then filtered off on a Büchner funnel, and washed with water, alcohol, and ether. This supposedly starch-free product yielded an extract with the cold hydrochloric acid (containing 21 grams of hydrogen chloride per 100 ml. of solution) which showed a very pronounced test for starch by iodine and produced a typical starch coagulum when poured into two volumes of alcohol. The obvious conclusion is that this new method recovers starch which escapes the official diastase method. Evidently this is starch which is so deeply imbedded in the bran coats that the treatment prescribed in the official diastase method does not dislodge it. Accordingly, specificity of this method for starch seems to be such that it recovers or determines all the starch as well as nothing but the starch. (This bran was finally rendered starch free, as judged by its failure to yield a starch containing extract with the hydrochloric acid, by being subjected to the diastase method a second time, so that it was exposed to a total of four treatments with malt diastase at 55°C., each of these treatments being preceded by gelatinization on the steam bath.)

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 119.

APPLICATION TO CEREAL PRODUCTS IN GENERAL.

The method has been applied to several cereal products, including wheat, wheat germ, patent flour, and oatmeal. Judged by the tests and criteria discussed in the preceding sections of this paper, all results have been satisfactory. In two instances the method was applied to products, viz., oatmeal and patent flour, whose starch contents had previously been determined by the official diastase method. The results by both methods are given in Table 2.

TABLE 2.

Results obtained by the official diastase method and the new method.

	DIASTASE METHOD *per cent*	NEW METHOD *per cent*
Oatmeal	58.75	60.34
	58.00	60.06
Patent Flour	62.84*	71.10
	64.31	70.58
	65.00	70.92

* Results of diastase method on patent flour are by C. E. Goodrich, Bureau of Chemistry, Washington, D. C.

Possible impurities in the starches coagulated by the new method cannot account entirely for the above differences in the results of the two methods. A more plausible explanation is that the results by the diastase method are low. One reason for suspecting these low results is that some of the starch may escape action by the diastase, as was shown to be the case of starch in bran. Another reason is that the hot hydrochloric acid used in the diastase method in the subsequent acid hydrolysis of the maltose into dextrose may destroy some carbohydrate material. In order to test this possibility starch was determined in the wheat starch listed in Table 1, the official direct acid hydrolysis method being used. The resulting duplicates were 97.2 and 97.3 per cent of dextrose. Multiplying the higher of these values, 97.3 per cent, by 0.9, the official factor for converting dextrose into its equivalent starch, 87.6 is obtained as the percentage of starch in the product. Or, multiplying the percentage of dextrose by the factor 0.93, which is sometimes recommended and used, the percentage of starch will be 90.5, as compared with 93.37, the result obtained by the new method and listed in Table 1. Noyes[1] (1904) and others have reported similar low results by the official methods for determining starch. It appears, therefore, that the results obtained by the new method represent more nearly true starch contents than do results obtained by the present official methods.

It is a pleasure for the writer to express here his thanks to Dr. E. V. McCollum for helpful suggestions in developing the method reported in this paper.

THE ESTIMATION OF CUPROUS OXIDE PRODUCED IN SUGAR ANALYSIS.

By CHARLES S. BISSON and J. GORDON SEWELL[2] (Division of Chemistry, Branch of the College of Agriculture, University of California, Davis, Calif.).

The volumetric method here presented for the determination of cuprous oxide, involving the use of standard solutions of potassium permanganate and ferrous sulfate, has been found to give concordant results. In this method the cuprous oxide is completely oxidized in acid solution with an excess of a standard solution of potassium permanganate. A measured excess of a standard solution of ferrous sulfate is then added to reduce the permanganate and any manganese dioxide that may have resulted from the interaction of potassium permanganate until the end point is reached.

The use of potassium permanganate to oxidize cuprous oxide has been studied by Caven and Hill[3] and made the basis of a method for the determination of cuprous oxide in sugar analysis. According to their procedure, the cuprous oxide obtained from the reduction of copper in Fehling's solution is partly filtered off on filter paper or asbestos and washed, and the remainder of the precipitate in the beaker is washed by decantation. The cuprous oxide on the filter and in the beaker is then dissolved in a measured excess of an acidified standard solution of potassium permanganate. After the cuprous oxide has dissolved, boiling water is added to raise the temperature to between 45° and 50°C., and the excess of potassium permanganate is determined by titration with a standard solution of oxalic acid. The method used by the writers avoids the difficulties mentioned by Caven and Hill, and, furthermore, it does not require that the titration be carried on in a hot solution.

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[1] *J. Am. Chem. Soc.*, 1904, **26**: 266.

[2] Valuable assistance was given by H. W. Allinger, analyst in the Division of Chemistry, who obtained some of the data presented.

[3] *J. Soc. Chem. Ind.*, 1897, **16**: 981; 1898, **17**: 124.

THE PERMANGANATE-FERROUS SULFATE METHOD.

SOLUTIONS.

The solutions used in this procedure are as follows: (1) A standard solution of potassium permanganate containing 3.16 grams of potassium permanganate per liter of solution, standardized with sodium oxalate; (2) a solution of ferrous sulfate containing 28 grams of ferrous sulfate ($FeSO_4\ 7H_2O$) and 10 cc. of 96 per cent sulfuric acid, sp. gr. 1.84, per liter of solution. Since this solution readily undergoes oxidation, its volumetric ratio with the permanganate solution should be determined for each series of analyses.

PREPARATION OF FILTER.

The filters used in this method consist of an asbestos filter pad formed on a perforated porcelain filter disc supported in a glass funnel. A bevel-edged perforated disc 25 mm. in diameter, supported in a 2.5 inch 60° short-stem funnel makes a convenient arrangement.

PREPARATION OF ASBESTOS FIBER.

The asbestos fiber used in the filters should be free from lumps or clots and should form a uniform suspension when shaken up with water. A fiber having the proper texture was prepared by floating ordinary long-fiber asbestos, such as is used for quantitative work, in a cylindrical glass percolator. By regulating the upward flow of the water through the percolator, a suitable form of asbestos was separated from the unshredded, lumpy material and collected in a large suction filter placed beneath the percolator. By further treating the asbestos prepared in this manner according to the procedure of the Association of Official Agricultural Chemists[1], a large quantity of fiber suitable for the process was readily prepared. The fiber thus prepared may be used repeatedly.

PROCEDURE.

After heating and boiling the Fehling solution with the sugar solution filter the mixture by means of suction through the filter prepared as described previously. Wash the cuprous oxide and beaker well with warm water (60°C.) to remove soluble substances. (It is not necessary to transfer all the cuprous oxide to the filter, as it may be washed by decantation in the beaker in which it was precipitated.) Remove the funnel from the suction flask, invert over the original beaker, shake out the pad and plate, and wash all cuprous oxide adhering to the funnel into the beaker with about 10 cc. of distilled water. Thoroughly disintegrate the pad by stirring with a glass rod. Add from a buret a quantity of the permanganate solution, in excess of that required to oxidize completely all the cuprous oxide; then add 10 cc. of 18 *N* sulfuric acid, sp. gr. 1 495, and stir the mixture until the precipitate is dissolved. (The quantity of permanganate solution added should be sufficient to impart a deep purple color to the solution.) While stirring the mixture, add from another buret 5 to 10 cc. of the ferrous sulfate solution in excess of that required to destroy the pink color of the permanganate. (At this point in the procedure no particles of unchanged cuprous oxide should remain in the mixture nor should any manganese dioxide adhere to the asbestos.) Add distilled water to increase the volume of the solution to 250 cc., and titrate this solution with the permanganate solution to the appearance of the usual end point. (If the quantity of copper is sufficient to impart a blue color to the solution after adding the ferrous sulfate, the color change at the end point will be from blue to lavender. The end point can be detected easily and the color does not fade any more rapidly than in the determination of iron with potassium permanganate.) Calculate the weight of cuprous oxide in grams from the following equations:

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[1] *Methods of Analysis, A. O. A. C*, 1925, 190.

$$(\text{Volume of } KMnO_4 - \text{Volume of } KMnO_4 \text{ equivalent to total volume of ferrous sulfate}) \times \frac{\text{Normal factor of } KMnO_4}{1000} \times \frac{143.1}{2} = \text{Weight of cuprous oxide.}$$

If the weight of copper represented by the titration is desired, the atomic weight of copper (63.57) should be substituted for the constant $\frac{143.1}{2}$ in the above equation. If the standard solution of potassium permanganate is 0.1000 N, one cc. of it will be equivalent to 0.00715 gram of cuprous oxide or to 0.006357 gram of copper. Reference to the Munson and Walker tables[1] will give the weight of the sugar equivalent to either cuprous oxide or copper.

RESULTS OF TITRATION EXPERIMENTS.

The procedure has been tested on sugar solutions prepared from pure dextrose and on those obtained from alcoholic extracts of plant material. The potassium permanganate solution was standardized with the U. S. Bureau of Standards sodium oxalate after heating according to their specifications.

The results of a series of analyses are given in Table 1. The cuprous oxide was produced by the action of pure dextrose on Fehling's solution when heated to boiling in 4 minutes and boiled 2 minutes. The precipitate, after being separated, was washed with water at 60°C., then with alcohol and finally with ether, and then dried at 100°C. for 30 minutes. The values given in Column 1 are those obtained for the weights of copper by calculation from the weights of cuprous oxide. In this series, varying quantities of the dextrose solution were used. Column 2 contains the values of the weight of copper for each analysis as calculated from the volume of the potassium permanganate used to oxidize

TABLE 1.

Results of gravimetric, volumetric, and electrometric procedures.

TRIAL	COLUMN 1	COLUMN 2	COLUMN 3
	Weight of Cu gravimetrically as Cu_2O	Weight of Cu by titration	Weight of Cu by electrolysis
	gram	*gram*	*gram*
1	0.1730	0.1734	0.1740
2	0.1678	0.1678	0.1682
3	0.1724	0.1736	0.1736
4	0.1662	0.1655	0.1660
5	0.1476	0.1475	0.1483
6	0.1451	0.1437	0.1447
7	0.1382	0.1380	0.1386
8	0.1454	0.1461	0.1468
9	0.3589	0.3597	0.3579
10	0.3594	0.3593	0.3580

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 434.

the cuprous copper according to the method outlined. Column 3 contains the weight of copper obtained by the electrolytic determination of the copper in the solutions resulting after titration. These solutions, after being filtered to remove asbestos, were gassed with hydrogen sulfide. The copper sulfide was removed by filtration and dissolved in nitric acid. The resulting solutions were electrolyzed according to the usual procedure.

The results given in Table 2 are from some experiments in which the method was applied to the determination of sugars in plant materials. In this series of experiments the cuprous oxide was not dried and weighed but was titrated directly according to the volumetric procedure. After titration, these solutions were treated as indicated in the other series of analyses and then electrolyzed to obtain the weight of copper. The values in Table 2 for the weight of invert sugar, equivalent to the weight of copper for each analysis, were obtained from the Munson and Walker tables.

TABLE 2

Results obtained on extracts of plant material.

TRIAL	WEIGHT OF Cu BY TITRATION	WEIGHT OF INVERT SUGAR EQUIVALENT TO WEIGHT OF COPPER BY TITRATION	WEIGHT OF COPPER BY ELECTROLYSIS	WEIGHT OF INVERT SUGAR EQUIVALENT TO WEIGHT OF COPPER BY ELECTROLYSIS
	gram	*gram*	*gram*	*gram*
1	0.0279	0.0141	0.0282	0.0142
2	0.0296	0.0149	0.0295	0.0148
3	0.0286	0.0144	0.0285	0.0143
4	0.0296	0.0149	0.0292	0.0147
5	0.0534	0.0269	0.0533	0.0268
6	0.0559	0.0281	0.0551	0.0277
7	0.0560	0.0282	0.0558	0.0281
8	0.0562	0.0287	0.0558	0.0281
9	0.0511	0.0257	0.0506	0.0255
10	0.0513	0.0258	0.0509	0.0256

NOTES ON THE PROCEDURE.

In the titration the sulfuric acid should not be added until the potassium permanganate solution has been added to the suspension of cuprous oxide, because sulfuric acid, as well as other oxygen acids, decomposes cuprous oxide into cupric oxide and copper. The cupric oxide dissolves readily in the acid, but, according to Proust[1] and Ehrenfeld[2], the copper is acted upon slowly by the potassium permanganate, and therefore the time required for the titration is greatly increased; moreover, as cuprous oxide in acid is readily oxidized by oxygen of the air to cupric salts, the results would be low.

Any unchanged cuprous oxide remaining after the addition of excess ferrous sulfate solution would be an indication that sufficient time was not allowed for dissolving the precipitate in the acid solution of potassium permanganate, and the presence of manganese dioxide might result from too low hydrogen-ion concentration or from failure to use sufficient excess of ferrous sulfate solution in decolorizing the permanganate solution.

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[1] *J. phys.*, 1800, 51: 182.

[2] *Z. anorg. Chem.*, 1908, 60: 208.

Although this method involves three steps in the titration process, namely, the oxidation of cuprous oxide with potassium permanganate, the reduction of the excess potassium permanganate with excess ferrous sulfate, and the final titration of the excess ferrous sulfate with potassium permanganate, it has the following advantages: (1) The oxidation of the cuprous oxide in the acid solution is rapid and complete; (2) the end point of the titration is easily detected and permanent; and (3) in case the end point in an analysis is over run, it can be easily determined by adding more ferrous sulfate solution and then titrating with potassium permanganate solution.

In determinations in which the weight of cuprous oxide is within the limits of the Munson and Walker tables, it is possible to prepare the filter, filter off and wash the cuprous oxide, and complete the titration according to this procedure within the 6 minute heating interval required in the method of Munson and Walker[1] for the determination of sugars. The procedure is applicable in all cases in which it is permissible to weigh the cuprous oxide.

CONCLUSION.

Cuprous oxide produced in the determination of sugars can be rapidly and accurately determined by this method.

The procedure does not require the use of expensive reagents, and large numbers of determinations may be made at comparatively small cost.

A STUDY OF METHODS PROPOSED FOR THE DETERMINATION OF THE UNSULFONATED RESIDUE IN PETROLEUM SPRAY OILS[2].

By J. J. T. Graham (Insecticide and Fungicide Laboratory, Bureau of Chemistry, Washington, D. C.).

Petroleum oils constitute an important class of insecticides, and their use is increasing. However, there is one serious objection to them—namely, that they may cause injury to foliage if applied in the growing season. An opinion has been expressed by workers in this field that this injury increases with the proportion of unsaturated hydrocarbons contained in the oil. In the absence of a practical rapid method for the determination of unsaturated hydrocarbons the percentage of the oil which sulfonates with strong sulfuric acid has been used as an index of their proportion. However, the sulfonation of an oil is an empirical procedure, and therefore it is important that a reliable method be adopted and used by all chemists. A number of variations in the procedure for sulfonation have been proposed.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 190.

[2] Read at the 42nd Annual Convention of the Association of Official Agricultural Chemists, Washington, D. C., October 18, 1926.

Gray and de Ong[1] recommend a procedure that is a modification of the method for the examination of coal tar creosotes adulterated with petroleum oils described by Bateman[2]. They use 37 *N* sulfuric acid and sulfonate for 1 hour at 95°-100°C., shaking at 10 minute intervals.

The Association of Official Agricultural Chemists has tentatively adopted a method requiring 38 *N* acid and five or six shakings during a sulfonation period of 10 minutes[3].

A modification of the Gray-de Ong method, differing chiefly in the temperature during the sulfonation, has been suggested by J. B. Terry of the Standard Oil Company of California[4]. In a later communication Terry suggested that in his method the temperature during the sulfonation be changed to 100°C., claiming that with this modification more concordant results are obtained. With this change the essential differences between his method and the Gray-de Ong procedure are the following: weighing of the charge of oil to be sulfonated, adding the acid in four portions, and reporting the results as percentage of unsulfonated residue rather than as percentage of sulfonated oils.

As Referee on Insecticides and Fungicides for the Association of Official Agricultural Chemists, the writer conducted collaborative tests of these methods, during the course of which he undertook additional investigation of certain points of the methods that appeared desirable.

EFFECT OF VARIATION OF THE TIME OF HEATING.

In the methods using 37 *N* acid the mixture of oil and acid is heated for 1 hour and shaken at 10 minute intervals. After each shaking the acid and oil quickly separate into two layers. In view of this fact it seemed probable that the reaction proceeds rapidly during the shaking, and stops when the two layers are well formed. To test this point sulfonations were carried out, samples of three different oils being used. The sulfuric acid was 37 *N*, and the bottles were maintained at a temperature of 65°C. The sulfonations were continued for periods of 60, 30, 24, 18, and 12 minutes, and the bottles were shaken for periods of 20 seconds at intervals of 10, 5, 4, 3, and 2 minutes, respectively. The mixtures in each case, therefore, were shaken to the same extent; the only difference in the procedure was a shortening of the intervals between the shakings. The results of these sulfonations are given in Table 1.

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[1] *Ind Eng Chem*, 1926, 18 175

[2] U S. Dept. Agr. Forest Service Circ 191 (1911)

[3] *This Journal*, 1926, 9. 136

[4] Personal communication to the writer

TABLE 1.

Effect of variation of time of heating.

37 *N* acid.

TIME OF HEATING	INTERVAL BETWEEN SHAKINGS	SAMPLE D UNSULFONATED	SAMPLE E UNSULFONATED	SAMPLE F UNSULFONATED
minutes	*minutes*	*per cent*	*per cent*	*per cent*
60	10	66.0	66.8	68.8
		65.6	66.4	68.8
30	5	65.2	66.4	68.8
		66.0	66.4	68.8
24	4	66.0	66.8	68.8
		65.6	66.4	68 8
18	3	65.6	66.8	68.8
		65.6	67.2	69.2
12	2	66.8	66.4	69.6
		67.2	66.4	68.4

Similar sulfonations were made with six oils, 38 *N* sulfuric acid at the same temperature being used. These sulfonations were continued for periods of 10, 20, 40, and 60 minutes and the shakings for 20 seconds at intervals of 2, 4, 8, and 12 minutes, respectively. In this, as in the previous experiment, every bottle received the same amount of shaking, only the interval between shakings being varied. The results of these sulfonations are given in Table 2.

TABLE 2.

Effect of variation of time of heating.

38 *N* acid

TIME OF HEATING	INTERVAL BETWEEN SHAKINGS	SAMPLE A UNSULFONATED	SAMPLE B UNSULFONATED	SAMPLE C UNSULFONATED	SAMPLE D UNSULFONATED	SAMPLE E UNSULFONATED	SAMPLE F UNSULFONATED
minutes	*minutes*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
10	2	66.0	68.0	64.4	58.0	57.6	60.0
		66.0	67.6	64.8	58.0	57.6	....
20	4	66.0	68.0	64.0	57.6	57.2	60.0
		66.0	68.0	64.4	58.0	57.6	59.2
40	8	66.4	68.0	64.4	57.6	57.6	60.0
		66.0	68.0	64.0	57.6	57.6	60.0
60	12	65.6	68.0	64.4	58.0	58.0	60.8
		65.2	68.0	64.4	58.0	58.4	60.8
						58.8	59.6

The physical characteristics of the oils used in the preceding experiments are given in Table 3.

TABLE 3.

Physical characteristics of the oils used.

SAMPLE	BASE	DENSITY 20°C	FLASH POINT	FIRE POINT	VISCOSITY 100°F SAYBOLT
A	Paraffine	0.883	360	400	110
B	Paraffine	0.886	385	445	153
C	Paraffine	0.896	335	390	113
D	Naphthene	0.924	345	380	298
E	Naphthene	0.919	335	365	200
F	Naphthene	0.912	305	335	105

The results in Tables 1 and 2 show conclusively that there is no advantage in long continued standing between shakings, and they indicate a lack of reaction after the separation into layers. The variations in the percentage of unsulfonated residue are well within the limits of repro-

TABLE 4.

Effect of variation in the amount of shaking.

38 *N* acid, period of heating, 10 minutes

PERIOD OF SHAKING	INTERVAL	SAMPLE A UNSULFONATED	SAMPLE B UNSULFONATED	SAMPLE C UNSULFONATED	SAMPLE D UNSULFONATED	SAMPLE E UNSULFONATED	SAMPLE F UNSULFONATED
seconds	*minutes*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5	1	66.4	67.6	64.4	58.8	58.8	60.0
		66.4	68.0	64.8	57.6	58.8	60.0
5	2	66.0	69.2	65.6	59.2	59.6	61.6
		66.4	68.8	66.0	59.2	59.2	61.2
5	5	67.2	69.2	65.6	60.8	60.0	62.0
		67.2	68.8	65.2	60.0	60.0	62.0
10	1	65.6	68.0	64.0	58.8	57.6	59.2
		65.2	68.0	64.0	58.8	57.6	59.2
10	2	66.4	68.4	64.0	58.4	58.4	60.8
		66.0	68.4	64.0	58.4	58.4	60.8
10	5	67.2	69.2	64.4	60.0	58.8	61.2
		67.2	69.2	65.2	60.0	59.6	61.2
20	1	66.0	68.8	64.8	57.0	57.2	59.0
		66.4	68.4	64.8	57.0	57.2	59.0
20	2	66.0	68.0	64.4	58.0	57.6	.
		66.0	67.6	64.8	58.0	57.6	60.0
20	5	67.2	68.4	65.2	58.0	58.0	60.0
		67.2	68.8	65.6	58.0	58.0	60.0

ducibility, and intervals of more than 2 minutes between shakings are therefore unnecessary. The methods requiring long total periods of sulfonation can be shortened without loss of accuracy.

EFFECT OF VARIATION IN THE AMOUNT OF SHAKING.

Sulfonations were made with 38 *N* acid and a constant heating period of 10 minutes. Shaking was continued for periods of 5, 10, and 20 seconds at intervals of 1, 2, and 5 minutes. The results are given in Table 4.

The results in Table 4 indicate that the percentage of unsulfonated residue is slightly greater in the case of the shorter total shaking periods. A constant amount of shaking, therefore, should be specified for any sulfonation method. The determination is empirical because no definite end point is reached.

These oils, after severe treatment with fuming sulfuric acid, will still show partial sulfonation when again treated with the 38 *N* acid. As an example, a sample of kerosene was heated on the steam bath with an excess of fuming sulfuric acid (84.1 per cent sulfur trioxide). The oil and acid were kept thoroughly mixed with a motor stirrer, and the heating was continued for 2 hours. After this exhaustive treatment the oil was separated and, when further treated, sulfonated to the extent of 2 per cent by the action of 38 *N* acid at 65°C. for 10 minutes.

CLARIFYING THE OIL COLUMN TO FACILITATE ITS MEASUREMENT.

After sulfonating and centrifugalizing some of the heavy oils the column of oil is so black that no line of demarcation between the oil and the acid is visible.

This condition usually occurs only with paraffine base oils of high viscosity. Gray and de Ong attribute this effect to carbonization caused by acid of too great a concentration and suggest that acids of greater concentration than 37 *N* are troublesome on this account. In the course of this work it was found that 37 *N* acid gave as much trouble as the 38 *N* acid, and indeed in some cases the 38 *N* acid gave a clearer oil.

The length of the heating period affects this blackening. The writer has made comparative sulfonations with a sample of oil that was especially prone to blacken, heating for 1 hour at 65°C. and 100°C., and for 18 minutes at 65°C. using 37 *N* acid, and for 10 minutes at 65°C., using 38 *N* acid. The sulfonations heated for 1 hour could not be read, while those heated for 10 and 18 minutes were sufficiently clear to give a satisfactory reading.

When excessive blackening occurs during the sulfonation there is no accurate method of determining the volume of the unsulfonated oil. A reading can be made by adding a few drops of water and allowing the bottle to stand a few minutes. The oil column will then rise above the water, and its volume may be read. Readings made in this way, however, are too high, possibly because of suspended colloidal material.

Dilution with hot sulfuric acid, and centrifuging while hot, was tried as a means of obtaining a clear oil column, but the results were negative.

The best method of sulfonation of oils likely to blacken is, as suggested by Gray and de Ong, to mix an oil of a known sulfonation value with the troublesome oil and to sulfonate the mixture. The writer modified this procedure somewhat by using kerosene that had been treated with an excess of fuming sulfuric acid for 2 hours. Five cubic centimeters of the troublesome oil was measured into an 18 gram 50 per cent cream bottle, and 5 cc. of the special kerosene was added; the mixture was then sulfonated with 20 cc. of 38 *N* acid for 10 minutes and shaken for 20 seconds at 2 minute intervals. This procedure gave a much clearer oil column than was obtained in straight sulfonations, and, in most cases, one that could be easily read with the assistance of a strong light.

The special kerosene used in the method described was slightly sulfonated, and the reading of this sulfonation was deducted from the total reading. Determinations made by this method on oils of known sulfonations checked the values obtained without the use of the diluent.

By the use of an inert oil, such as the special kerosene, the full strength of the acid is allowed to react with the unknown oil, and a more correct value is obtained than would be the case if both oils were being sulfonated simultaneously.

A PROPOSED METHOD OF SULFONATION.

The following improved procedure is proposed for the determination of the unsulfonated residue in petroleum spray oils:

> With a pipet, measure 5 cc. of the oil into a Babcock cream bottle. (After preliminary draining, in the case of heavy oils, to reduce the viscosity warm the pipet by drawing it several times through the flame of a Bunsen burner and then drain thoroughly). In lieu of this procedure, determine the density of the oil and weigh the equivalent of 5 cc. Use a bottle about 15 cm. long—either the 9 gram 50 per cent or the 18 gram 30 per cent cream bottle. Add slowly 20 cc. of the 38 *N* fuming sulfuric acid, gently shaking or rotating the bottle, taking care that the temperature does not rise above 60°C., and cooling in ice water if necessary. When the mixture no longer develops heat on shaking, agitate thoroughly, place the bottle in a water bath, and heat at 60°-65°C. for 10 minutes, keeping the contents of the bottle thoroughly mixed by shaking vigorously for a period of 20 seconds at 2 minute intervals. Remove from the bath and fill the bottle with concentrated sulfuric acid until the oil rises into the graduated neck. Centrifugalize for 5 minutes (or longer if necessary to obtain a constant volume of the oil) at 1200–1500 revolutions per minute. Read the volume of unsulfonated residue from the graduations on the neck of the bottle, and from this reading calculate the percentage by volume of the unsulfonated oil.

SUMMARY.

In a study of methods for the determination of unsulfonated residue in mineral oils it is shown that the reaction takes place almost entirely during the periods of shaking and that an interval of more than 2 minutes between shakings is unnecessary.

The quantity of unsulfonated residues is shown to be slightly greater in the case of short total shaking time.

An improved method is proposed to facilitate reading of the volume of the oil column in the sulfonation of oils that give a black residue when sulfonated.

A method is proposed for the determination of unsulfonated residue in which the time of shaking and the length of the interval between shakings, as well as the length of the reaction period, are definitely stated.

ERRORS IN ANALYSIS OF ALKALOIDS CAUSED BY PRESENCE OF FATTY ACID OR SOAP[1].

By H. R. WATKINS and S. PALKIN (Drug Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture).

The determination of alkaloids in low-grainage compressed tablets is often difficult. One of the most troublesome features is that when the quantity of excipient is very large and fatty acid is present the ordinary procedure of suspending the tablet material in aqueous alkaline medium for extraction with an immiscible solvent results in the formation of difficult emulsions, making complete extraction tedious and often doubtful.

Aside from the incomplete extraction caused by such emulsions, appreciable errors of an entirely different character may result from the soap itself and give rise to alkaloidal values erroneously high. Soaps of magnesium and calcium dissolve to some extent in chloroform, are carried along with the alkaloid, and ultimately enter into the titration as that much additional base. Therefore, if an excipient in which soap or fatty acid has been used contains, in addition, some magnesia or calcium carbonate (possibly from impure talc), calcium or magnesium soap is formed during analysis and dissolves to some extent in the chloroform during the alkaloidal extraction, as indicated. This does not occur with potassium or sodium soap. It might be supposed that simultaneous extraction of fatty acid with alkaloid would result in partial neutralization and a low assay, but such was not found to be the case. Extraction of the alkaloid from an ammoniacal solution results in the simultaneous extraction, not of an ammonium soap (such as ammonium stearate), but of the fatty acid itself, evidently as a result of the hydrolysis of the soap. This acid, in the normal process of alkaloidal titration (methyl red indicator), exists merely as an insoluble pasty mass on the sides of the vessel and does not affect the titration. This deposit does not occur in the case of sodium soaps, at least when small quantities of such soaps accompany the alkaloidal solution.

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[1] Presented before the section of Chemistry of Medicinal Products at the Philadelphia meeting of the American Chemical Society, September, 1926, and published here by courtesy of *Industrial and Engineering Chemistry*.

EXPERIMENTAL.

A typical case in which this problem presented itself was in the analysis of 1/200 grain tablets of atropine sulfate. On examination of the excipient this was found to contain stearic acid and compounds of magnesium and calcium.

Table 1 gives the results of analysis in which the method used was the direct extraction with chloroform of a suspension of 200 tablets in water made alkaline with ammonia. The acid solutions of the atropine sulfate obtained from the chloroformic extracts for titration were found to contain a deposit of soapy or waxy material on the walls of the titrating vessel. As will be observed the results thus obtained were exceedingly variable.

TABLE 1.

Analysis of 1/200 grain atropine sulfate tablets.

(200 tablets used in each determination—A. O. A. C. Method)*

ANALYST	ATROPINE SULFATE FOUND†	PROPORTION OF DECLARED AMOUNT
	mg.	*per cent*
1	74.32	115.1
	75.23	116.1
	72.71	114.7
2	65.49	101.0
	67.57	104.3
3	56.63	87.4
	55.86	86.2

* *Methods of Analysis, A O. A C*, 1925, 390.
† Calculated from titration of extracted base.

As further difficulties may arise in the case of atropine because of instability under certain conditions, precautions were taken to insure against hydrolysis of the atropine as described in a previous publication on "The stability of atropine and hyoscyamine"[1]. As pointed out in that paper, it is possible by using care to keep these alkaloids intact and to handle them safely throughout an analytical procedure. To prevent the free alkaloid from being in neutral or alkaline water solution or even

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[1] *J. Am. Pharm. Assoc.*, 1927, 16: 21.

TABLE 2.

Experiments with 1/200 grain atropine sulfate tablets.

(200 tablets used in each analysis.)

ANALYST	0 02 N ACID CONSUMED					ATROPINE SULFATE (CALCULATED)					PROPORTION OF DECLARED AMOUNT				
	Fat not removed*	After removal of fat*	Reextraction of titrated solutions†			Fat not removed*	After removal of fat*	Reextraction of titrated solutions†			Fat not removed*	After removal of fat*	Reextraction of titrated solutions†		
			1	2	3			1	2	3			1	2	3
	cc	*cc.*	*cc*	*cc*	*cc*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
W	10.73	8.55	8.55	8.6	8.55	74.57	59.4	59.4	59.74	59.4	115.1	91.7	91.7	92.0	91.7
S	9.8	8.45	8.45	8.45		68.07	58.7	58.7	58.7	..	105.04	90.5	90.5	90.5	....
M	8.98	8.51		. .	.	62.38	59.1	. .	.		96.3	91.2	.		.
P	9.84	8.5	8.5	.	.	68.34	59.04	59.04	.	.	105.4	91.1	91.1		.

* Fat is essentially in form of free fatty acid.

† Each succeeding "reextraction" represents the quantitative extraction of atropine from the titrated solution of the determination directly preceding.

in contact with water, especially at elevated temperature, is of primary importance.

In order to obviate the difficulty caused by the formation of emulsions, experiments were made in which the alkaloid was removed from the bulk of the excipient and subsequently extracted and determined as described as a means of obtaining a clear oil column, but the results were negative. under Method A. Results thus obtained by four analysts (Table 2) are variable and, with one exception, well above the declared amount. Soapy deposits accompanied the titrated solution, as in the previous experiments.

Extraction of these titrated alkaloidal solutions with chloroform (after the addition of excess acid) yielded fat-free alkaloidal solutions. When made alkaline with ammonia and again extracted with chloroform with subsequent evaporation of the solvent, these solutions gave alkaloidal titrations considerably lower than before but the results obtained by the different analysts were uniform. As shown in Table 2, the atropine sulfate content was found to be consistently below the amount declared on the label.

That this decrease in alkaloid titrated is not due to losses incident to the process of analysis is evidenced by the results as shown in Table 2. Repeated reextraction and retitration of the alkaloid in exactly the same manner as for an original determination gave identical results.

The reasons for this apparently paradoxical behavior of stearic acid in causing high instead of low alkaloidal titrations are brought out in the following series of experiments (Table 3).

Soaps were prepared from stearic acid (also from the original fat obtained from the tablets), (a) ammonia, (b) sodium hydroxide, or (c) potassium hydroxide being used as a base; 83.5 mg. of atropine (representing approximately 100 mg. of atropine sulfate) was dissolved in a slight excess of acid, small quantities of the prepared soaps were added, and the whole was made alkaline with ammonia. The alkaloid was extracted with chloroform and determined in the usual way. In all cases the atropine was completely recovered. No soap or fatty acid was present in the titrated solution of (b), but fatty acid was present in (a), showing that the ammonium soap was easily hydrolyzed and fatty acid extracted along with the atropine. This condition, however, had no effect on the accuracy of the alkaloid determination.

With potassium stearate such difficult emulsions were obtained that complete extraction of the alkaloid was impossible, and the emulsions did not yield to the centrifuge. Removal of fatty acid before alkaloid extraction was therefore essential.

When calcium and magnesium soaps (prepared as already described) were incorporated with weighed quantities of atropine (83.5 mg.) and extraction was made as described, very high results were obtained. After removal of the fatty acid, accurate recovery of the atropine was effected.

TABLE 3.

Experiments with ammonium, sodium, potassium, calcium, and magnesium stearates.

(83.5 mg. of atropine used in each experiment.)

SOAP USED	EXTRACTED BASE CALCULATED AS ATROPINE			
	In presence of soap	Recovery	After removal of stearic acid from titrated solution	Recovery
	mg.	*per cent*	*mg.*	*per cent*
Ammonium stearate	83.03	99.44		
	84.07	100.6	83.49	99.99
Sodium stearate	83.49	99.99		
	84.07	100.6	83.49	99.99
Potassium stearate	72.8*	87.2*	81.87	98.07
	82.33*	98.61*	83.78	100.33
			83.49	99.99
Calcium stearate	85.52	102.44		
	86.96	104.14	83.49	99.99
	107.6	128.8	84.07	100.6
Magnesium stearate	86.96	104.14		
	86.67	103.8		
	122.26	146.4	83.61	100.1
	107.04	128.2	83.6	100.1

* Potassium soap caused more difficult emulsions than other soaps and made complete extraction of atropine impossible.

In a series of variable results from determinations in which emulsions give trouble, it is ordinarily assumed that incomplete extraction will follow and that the higher results are therefore more nearly accurate. As shown in the experimental data both on the original tablets and on synthetic mixtures, a real source of error exists even where emulsions are eliminated and full precautions to preserve the alkaloid are taken. Such errors may give results far above the true content of alkaloid.

The following procedure is suggested for alkaloidal tablets of low grainage where unworkable emulsions are produced or when soap or stearic acid is present.

Method A.

A convenient number of the tablets (a quantity representing about 65 mg. of alkaloidal sulfate), either in the form of a finely ground powder or in the form of the tablets as such, are moistened with a little water in a small mortar (preferably glass), 1 cc. of normal sulfuric acid is added, and the whole is triturated to form a fine, creamy mass. This mass is diluted further and completely transferred to a beaker, care being taken to keep the whole aqueous volume from being much over 40 or 50 cc. An equal volume of alcohol is added with stirring, and the mixture is filtered by suction, a bell-jar arrangement with a funnel and plate being preferred, and the filtrate is collected in a 200 cc. Erlenmeyer flask. The residue is washed with a little 50 per cent alcohol. The filtrate is evaporated on the steam bath to a small volume by using an air blast. The insoluble excipient is completely transferred to the original beaker, and the process of extraction with water-alcohol mixture, filtering, and washing is repeated. This second filtrate is added to the original water-alcohol solution, and the whole is evaporated on the steam bath to about 25 or 30 cc. The cooled concentrate is then completely transferred to a separatory funnel, 2 cc. of 5 *N* ammonia is added, and the solution is immediately extracted with chloroform in the usual way, about four extractions being made, the first, with an approximately equal volume of chloroform. The chloroformic extracts are washed successively with a little water, and the combined chloroformic solutions are evaporated on the steam bath to 5 or 10 cc. (*not to dryness*). A quantity of 0.02 *N* acid (in slight excess) is added, and the whole is heated on the steam bath to remove the remainder of chloroform, an air blast being used preferably. The back titration is then carried out in the usual way.

Method B.

If a soapy deposit is present in the titrated solution, removal of the fatty acid and subsequent reextraction of the alkaloid are carried out as follows: To the titrated solution, a few cc. of 0.1 *N* acid is added. The whole is completely transferred to a 200 cc. separator and, to remove fatty acid, extracted twice with chloroform (20 cc. and 15 cc. portions). Before being discarded, the chloroformic solutions are washed once with about 10 cc. of slightly acidulated water, and the wash water is added to the main alkaloidal solution. The alkaloidal solution is then made ammoniacal and extracted with chloroform, the necessary precautions being taken against hydrolysis in the case of atropine or hyoscyamine, as already described.

CONCLUSIONS.

When alkaloid is extracted from aqueous solution in the presence of soap, titration of the extracted residue (methyl red indicator) may not represent the true alkaloidal content. In the presence of calcium and magnesium soaps present originally or formed in the process of analysis a considerable quantity of the soap may follow along with the alkaloid and enter into the titration as that much additional base. In the presence of ammonium and sodium soaps while some fatty acids may be carried along with the alkaloid as a result of soap hydrolysis these fatty acids do not affect the titration. Removal of the fatty acid from the acidified solution containing the alkaloid, therefore, is essential for quantitative alkaloid determination, not because the fatty acid will cause low results, but for the reason that soaps of calcium or magnesium may form and render the results high.

THE SIGNIFICANCE OF THE SOLUBILITY AND "ACTIVITY" OF THE NITROGEN IN COCOA (CACAO) BY-PRODUCTS[1].

By G. P. Walton and R. F. Gardiner (Bureau of Soils, Department of Agriculture, Washington, D. C.).

In the interpretation of results obtained in the chemical examination of fertilizer material the principal criterion of the availability or fertilizer value of the nitrogen, phosphoric acid, or potash is the relative solubility in water of the constituent, or the relative ease with which it may be converted into a water-soluble form. Solubility tests of availability are applied to the nitrogen of organic residues as well as to fertilizer nitrogen of mineral origin, on the well-founded assumption that the nitrogen must eventually be brought into solution before it can be converted into a form that can be utilized by growing plants.

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[1] Read before the Fertilizer Section, American Chemical Society, at Philadelphia, Sept. 7, 1926. An abstract was presented at the meeting of the Association of Official Agricultural Chemists, Oct. 18, 1926. Published through the courtesy of *Industrial and Engineering Chemistry*.

Conversely, the water-soluble nitrogen of recognized fertilizer materials, including the soluble nitrogen of seed residues and other organic ammoniates, has been generally accepted as being available plant food[1]. C. S. Robinson has pointed out comparatively recently, however, that the availability of the water-soluble nitrogen of organic materials has not been proved by experimental investigation[2]. Data reported in this paper, obtained in an investigation of the solubility and nature of the nitrogen of cocoa by-products used as fertilizer materials, illustrate the force of Robinson's observation. In view of the large quantities of the cocoa by-products that have been available and used in fertilizer, it is thought that these data may be of more than mere theoretical interest.

COCOA BY-PRODUCTS EXAMINED.

The investigation of cocoa by-products, of which the chemical work on the nitrogen formed a part, was undertaken by the writers following the production in this country of large quantities of by-product cocoa press-cake and solvent-defatted cocoa, and their appearance in the market for fertilizer materials.

Both of these residues are by-products of the preparation of cacao butter. The press-cake results from the hydraulic pressing of cacao beans, following roasting, decortication, and grinding. The defatted cocoa is the residue remaining after subjecting the by-product press-cake to extraction with benzene for the recovery of additional cacao fat.

Details regarding the processes of manufacture and the different types of by-product are contained in a recent bulletin of the Department of Agriculture, entitled "Cocoa by-products and their utilization as fertilizer materials".[3] The conditions in the cocoa and chocolate industry in this country, particularly the change in the market for cacao butter that has made it profitable to press cacao beans for the butter alone and has given rise to the production of by-product press-cake and extracted cocoa are also discussed in the same bulletin.

PRODUCTION OF COCOA BY-PRODUCTS.

On the basis of the production for 1924 the annual production of by-product cocoa cake is estimated at 25,000 tons. The two main types of cake made are the more common with higher fat content and the dry-pressed cake containing a decidedly lower percentage of fat, which is turned out by powerful presses. The production data for solvent-extracted cocoa cannot be given, but the quantity has recently been sufficiently large to account for the disposal of an important part of the higher-fat press-cake. In addition to these cocoa by-products about 20,000 tons of the shells are annually produced in the United States, and this by-product has long been a regular article of commerce in the form of cocoa-shell meal. Large quantities are used as conditioning material in the preparation of mixed fertilizers. Quantities of the by-product press-cake, also, have eventually been consumed in fertilizers, but mainly in the form of the defatted residue after solvent extraction.

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[1] Cf. Jones, C. H. *J Ind Eng. Chem.*, 1912, 4: 430.
[2] *This Journal*, 1924, 7: 375
[3] U. S Dept Agr Dept. Bull 1423

LABORATORY INVESTIGATION.

Representative samples of the by-product cake—both ordinary and dry-pressed—of the solvent-extracted cocoa, and of commercial cocoa shells were analyzed and also examined as to the solubility and quality of their nitrogen. The average analytical results, showing the data pertaining to the fertilizer value of the material for each type of cocoa by-product, are given in Table 1.

TABLE 1

Average proximate analyses of cocoa by-products.

COMMERCIAL MATERIAL REPRESENTED		MOISTURE	ETHER EXTRACT (CRUDE FAT)	ASH	AMMONIA NH_3	PHOSPHORIC ACID P_2O_5	POTASH K_2O	THEOBROMINE AND CAFFEINE ALKALOIDS
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
By-product press-cake	Ordinary*	3.4	19.9	5.4	4.7	1 3	1.9	2.8†
	Dry-pressed‡	3.2	12.5	6.4	5.0	1.7	2.0	3.2§
Solvent-extracted cocoa, dried**		6.2	2.6	9 0	5.4	1 8	2.2	3.2§
Cocoa-shell meal†		4.1	9.2	8.2	3.5	1.2	2.4	1 8

* Average for 6 samples.
† Average for 2 samples
‡ Average for 4 samples
§ For 1 sample
** Average for 3 samples

By-product cocoa press-cake may contain from as little as 9 per cent of fat (in cake pressed very dry) to over 20 per cent. The average nitrogen content is 4 per cent, although 4.5 per cent of nitrogen was found in one dry-pressed cake. Calculated to a moisture-free, fat-free basis, the percentage content of nitrogen in the press-cakes and solvent-extracted cocoas was found to be fairly uniform, averaging just under 5 per cent.

It will be seen that the by-product cocoa cake is not unlike poorly pressed castor pomace in proximate composition, except that for corresponding fat contents the percentage of nitrogen in the cocoa by-product runs about 1 per cent under that in castor pomace.

The content of theobromine and caffeine alkaloids[1], ranging from 2.7 to 3.2 per cent for the press-cakes and extracted cocoa and 1.6 to 2 per cent for the shells, is sufficiently high to be a factor in the disposal of the by-products. For example, it has to be considered in the utilization of the shells or cake in the feeding of live stock. In the light of past investigations it appears that the alkaloid content should also be considered in relation to the fertilizer value of these materials.

Results of European experiments reported by Schreiner and Skinner[2] showed theobromine to be harmful to radishes in water cultures and caffeine to be injurious to a number of plants, including onions, celery, and corn in water or sand cultures. In view of these findings and in the absence of experimental evidence as to the behavior of the alkaloids in the soil, the fertilizer value of the nitrogen represented by the alkaloids in the cocoa materials is open to question.

SOLUBILITY OF THE ALKALOIDS.

The solubility of the alkaloids in samples of press-cake and dried extracted cocoa was tested mainly for the purpose of determining whether or not the alkaloid nitrogen is included with the water-soluble nitrogen, and hence ordinarily credited with being available. Five grams of the sample was leached with 625 cc. of distilled water at room temperature, and the content of total alkaloid in the undissolved residue was determined. It was found that from 94 to 99 per cent of the total alkaloid dissolved, and it may be assumed, therefore, that practically all the alkaloid nitrogen is included with the water-soluble nitrogen as determined by the official method.

The theoretical nitrogen content of the alkaloids occurring in the cocoa by-products is 31 per cent, since previous work has shown that theobromine constitutes about 95 per cent and caffeine only 5 per cent of the total alkaloids. According to Wadsworth[3], however, not all of this nitrogen appears in results obtained by the Kjeldahl type of method for the determination of nitrogen. In no case did he obtain more than 94.8 per cent of the theoretical nitrogen content of theobromine. In this paper, therefore, the values reported for alkaloid nitrogen content have been placed at 94.8 per cent of the theoretical.

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[1] The determinations of theobromine plus caffeine were made in the Drug Control and Pharmacognosy laboratories of the Bureau of Chemistry by a carefully tested method involving suspension of the material in weakly acidified water, extraction of the alkaloids with chloroform, and final purification of the alkaloids by sublimation.

[2] U. S. Dept. Agr. Bur. Soils Bull. 87, pp. 72, 78.

[3] *Analyst*, 1921, **46**: 32.

TABLE 2.

Average data relating to the solubility and quality of the nitrogen in cocoa by-products.

BY-PRODUCT REPRESENTED	TOTAL NITROGEN *N*	ALKALOID NITROGEN*		WATER-SOLUBLE NITROGEN†		WATER-INSOLUBLE NITROGEN				
		In samples	As percentage of water-soluble nitrogen†	In samples	As percentage of total nitrogen	In samples	Soluble in neutral permanganate solution	Distilled from alkaline permanganate solution	"Activity", as percentage of water-insoluble nitrogen	
									By the neutral permanganate method	By the alkaline permanganate method
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
By-product cocoa press cake‡	3.97	0.85§	56.4§	1.48	37.3	2.49	1.44	0.54	57.8	21.7
Solvent-extract cocoa, dried**	4.36	0.94††	57.7††	1.62	37.2	2.74	1.26	0.54	46.0	19.7
Cocoa-shell meal‡‡	2.86	0.53	53.0	1.00	35.0	1.86	0.79	0.32	42.5	17.2

* The alkaloid nitrogen determinable by the Kjeldahl method, amounting to about 95% of the theoretical content

† Practically all the determinable alkaloid nitrogen is included with the water-soluble nitrogen.

‡ Average for 8 samples—4 ordinary and 4 dry-pressed cakes.

§ Average for 3 samples.

** Average for 4 samples.

†† For 1 sample.

‡‡ Average for 2 samples.

SOLUBILITY AND QUALITY OF COCOA NITROGEN.

The average results obtained in the chemical investigation of the nitrogen of each of the three cocoa by-products are shown in Table 2. A little more than one-third of the total nitrogen of each of the cocoa by-products is water-soluble, as determined by the official method. This is a relatively large share of soluble nitrogen for organic ammoniates, and the absolute amount of soluble nitrogen, ranging from 0.8 per cent in one of the shell samples to over 1.8 per cent in an extracted cocoa also is comparatively high.

Between 50 and 58 per cent of the water-soluble nitrogen must be alkaloid nitrogen, however, since practically all the theobromine and caffeine were found to be soluble, and it is questionable whether this alkaloid nitrogen has any plant-food value. On the contrary, even in the soil the soluble alkaloids represented by this nitrogen may be harmful to growing plants.

"ACTIVITY" OF THE WATER-INSOLUBLE NITROGEN.

The water-insoluble nitrogen of the cocoa by-products must be classed as of inferior quality on the basis of the average "activity" values obtained by either the neutral or alkaline permanganate method. Although no limit has been officially fixed for either permanganate method for defining acceptable activity for the water-insoluble nitrogen of crude or unmixed organic fertilizer materials, it is commonly considered that the passing marks for the insoluble nitrogen in such materials should be about the same as the limits that have been officially adopted for the interpretation of results on mixed fertilizers[1]. The minimum acceptable activity for the insoluble nitrogen of mixed fertilizers has been fixed at 80 per cent for the neutral and 50 per cent for the alkaline permanganate method.

It is apparent that the neutral permanganate method assigns a higher activity to the insoluble nitrogen of the cocoa products relative to the respective passing percentages than the alkaline method does. As a matter of fact, a recent examination of two samples of dry-pressed by-product cocoa cake showed "activities" of the water-insoluble nitrogen above 80 per cent by the neutral method, which classed the materials as satisfactory sources of nitrogen, whereas the alkaline method gave activity values of less than one-half of the acceptable limit of 50 per cent. It is not uncommon to encounter fertilizers with insoluble organic nitrogen exhibiting satisfactory activity according to one method and failing to show acceptable activity by the other, but some of the cocoa by-products showed greater discrepancies between the values obtained by the two permanganate methods than have been observed in the literature for any material. (See results obtained by Hartwell and Pember[1].)

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[1] *This Journal*, 1925, 8: 250. Compare also Robinson, *loc. cit.*

NECESSITY FOR STRICT ADHERENCE TO OFFICIAL METHODS.

Because of unexplained variation in some previous analytical results, the two samples of dry-pressed cake were examined further and used for a brief investigation of the effects of slight deviations from the neutral permanganate method as officially defined.

Both samples were carefully prepared. They were ground to pass a 40-mesh sieve and thoroughly and uniformly mixed. Repeated determinations showed that they contained 4.46 and 4.40 per cent of total nitrogen, and 3.01 and 2.79 per cent of water-insoluble nitrogen, respectively. The results obtained from different sets of determinations were highly consistent.

The water-insoluble nitrogen insoluble in neutral permanganate was found to average 0.31 and 0.49 per cent, respectively, when the official instructions were followed to the letter. (In determining the residual-nitrogen by the Kjeldahl method, after the permanganate digestion, it has been found advisable to add an extra 100 cc. of distilled water before starting the final distillation. Otherwise, the presence of large quantities of manganese in the flasks causes severe bumping before the distillation of ammonia is complete.) Two sets were run, at different times, and the maximum variation between individual determinations was 0.05 and 0.09 per cent for the samples. The average activity by the strictly "official" method was 89.7 and 82.4 per cent, respectively.

The official procedure was then varied by dissolving the 1 gram of sodium carbonate in the 100 cc. of 2 per cent permanganate solution and adding the combined reagents to the water-insoluble residue and 25 cc. of tepid water, instead of first adding the sodium carbonate in the dry form. It was supposed that this innovation, which would facilitate the procedure by avoiding separate weighings of carbonate for each individual charge, would yield lower activity values. This proved to be the case, but the difference was hardly great enough to be significant.

The regular procedure was then changed merely to the extent of drying the insoluble residues from the water extraction overnight at 70°C. If this innovation were permissible, larger sets could be run, because the preparation of the water-insoluble, permanganate-insoluble residue could be spread over two days. It was found that this drying seriously affected the solubility of the water-insoluble nitrogen in the permanganate solution. The results obtained on the two samples averaged 0.70 and 0.73 per cent of permanganate-insoluble nitrogen, respectively, with extreme variation between individual determinations of 0.09 and 0.10 per cent. Hence, this preliminary drying of the water-insoluble residues decreased the "activity", as measured by the neutral permanganate method, from 89.7 to 76.7 per cent for the first sample, and from 82.4 to 73.8 per cent for the second. In other words, these two samples of by-product cocoa passed the test when the official instructions were exactly followed, but they failed to pass when the method was altered to the slight extent of drying the water-insoluble residue at 70°C. prior to the permanganate digestion.

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[1] *This Journal*, 1923, 7. 55.

CONCLUSIONS.

Not all water-soluble, organic nitrogen in fertilizers can be freely accepted as being available plant food. More than one-third of the nitrogen of cocoa by-products is soluble in water, but over one-half of this soluble nitrogen occurs as a constituent of the alkaloids theobromine and caffeine, and these alkaloids may be not only without plant-food value, but they may even be injurious to growing plants.

The water-insoluble nitrogen of cocoa by-products may exhibit acceptable "activity" by the neutral permanganate test, while its activity measured by the alkaline method may be less than one-half of the "passing" value.

The "activity" of insoluble organic nitrogen, as measured by either permanganate method, is an empiric value; correct or significant results can be obtained only by the strictest adherence to the spirit as well as to the letter of the official instructions for conducting these determinations.

Certain portions of the official instructions, such as the temperature of the bath for the neutral permanganate digestion and the volume of water to be added prior to the final Kjeldahl distillation of the residual ammonia, should be stated more explicitly.

FIRST DAY.

MONDAY—MORNING SESSION.

REPORT ON WATERS, BRINE, AND SALT.

By C. H. **Badger** (Bureau of Chemistry, Washington, D. C.), *Referee.*

It is recommended[1]—

(1) That the following corrections be made:

(*a*) In the official method for the determination of manganese by the bismuthate method, *Methods of Analysis, A. O. A. C., 1925*, Chapter VIII, Section **75**, lines 8 and 17, change the word "bisulfate" to "bisulfite".

(*b*) In Method I for the determination of iodine in the presence of chlorine and bromine, *Methods of Analysis, A. O. A. C., 1925*, Chapter VIII, Section **106**, change the last two sentences to read as follows: "Since one-sixth of the iodine titrated represents the quantity originally present, one cc. of the 0.05 *N* sodium thiosulfate solution used is equivalent to 1.058 mg. of iodine".

(2) That the referee study methods for the analysis of salt with particular reference to the determination of ingredients added to prevent caking.

REPORT ON TANNING MATERIALS AND LEATHERS.

By T. D. **Jarrell** (Leather and Paper Laboratory, Bureau of Chemistry, Washington, D. C.), *Referee.*

The work undertaken this year was a collaborative study of the determination of moisture in leather. This report includes results both by members of this association and of the American Leather Chemists' Association. All the work was done on the same set of samples and according to the same outline. The results by the members of the A. L. C. A. were reported at the last annual meeting of that association in June, 1926[2]. They are quoted here to show all the work that has been done under these directions.

The following chemists cooperated in the work:

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**: 60.
[2] *J. Am. Leather Chem. Assoc*, 1926, **21**: 436

A. O. A. C. Members.

L. E. Bopst, University of Maryland, College Park, Md.
M. P. Etherege, Mississippi Agricultural and Mechanical College, Miss.
O. B. Winter, Agricultural Experiment Station, East Lansing, Mich.
J. H. Mitchell, Agricultural Experiment Station, Clemson College, S. C.
W. D. Edington, Bureau of Chemistry, Washington, D. C.
T. D. Jarrell.

American Leather Chemists Association Members.

L. A. Cuthbert, Elk Tanning Co., Ridgway, Pa.
V. J. Mlejnek and K. H. Knight, The Graton & Knight Mfg. Co., Worcester, Mass.
A. C. Orthmann and C. C. Kaplan, Pfister & Vogel Leather Co., Milwaukee, Wis.
R. N. Porter, Ashland Leather Co., Ashland, Ky.
F. F. Marshall, Kistler Leather Co., Lock Haven, Pa.

W. D. Edington and T. D. Jarrell also participated in the A. L. C. A. collaborative work.

The work included a study of three methods for determining moisture in leather: (1) drying in an electric air oven at 100°C. for five successive 5-hour periods, using a regular ground-glass stoppered weighing bottle; (2) drying in a current of dry air in the same oven and at the same time as (1) for five successive 5-hour periods, using a special ground-glass stoppered weighing bottle devised by Veitch and Jarrell[1]; and (3) by the Bidwell-Sterling[2] modification of the toluene distillation method.

Before sending out instructions and samples to collaborators preliminary work was done by the referee on the toluene distillation method with the idea of standardizing the details of the procedure.

The following directions, together with the two samples of leather mentioned therein, were sent to those collaborating:

DIRECTIONS TO COLLABORATORS ON METHODS FOR THE DETERMINATION OF MOISTURE IN LEATHER, A. O. A. C., 1926.

Sample No. 1.—Vegetable Tanned Sole Leather.
Sample No. 2.—Black Greased Harness Leather.

Do not open the samples until ready to weigh out and keep bottles closely sealed between weighings. It is important to weigh all charges for each method at the same sitting, because when the containers are once opened the leather may change considerably in moisture content.

If possible follow closely the procedure outlined, indicating any deviation from it in your report.

All the data and comments upon the different methods should be reported to the referee not later than July 31, 1926.

WEIGHING CHARGES.

Weigh rapidly and accurately four 5 gram portions of Sample No. 1 and place into previously dried and weighed ground-glass stoppered weighing bottles—Charges 1 and 2 in regular bottles measuring 45 mm. wide and 65 mm. high, and Charges 3 and 4 in special weighing bottles measuring 45 mm. wide and 85 mm. high and which have tops like the one shown in Fig. 1 of the Report on the Determination of Moisture in Leather in 1925[1]. Place all these bottles into a desiccator that contains no desiccating agent in order to eliminate as nearly as possible a change in weight. Determine moisture in these charges by the oven-drying method included in these directions.

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[1] *J. Am. Leather Chem. Assoc.*, 1925, 20: 334.
[2] *This Journal*, 1925, 8: 295.

At the same sitting also weigh rapidly and carefully two 20 gram portions of the same sample (No. 1) and transfer them to 500 cc. Erlenmeyer flasks. Immediately cover with dry toluene (about 200 cc.) and connect to the Bidwell and Sterling moisture tube as shown in their method[2]. Determine moisture at once by the toluene distillation method included in these directions. Number these charges 5 and 6

OVEN DRYING METHOD.

(Charges 1 to 4 inclusive.)

On the same day that the charges are weighed out or as soon thereafter as possible place Charges 1, 2, 3, and 4 in an electric air oven maintained at exactly 100°C. and dry for five successive 5 hour periods (25 hours) at this temperature. Remove the tops of the regular weighing bottles (1 and 2) during the drying periods. Immediately and as rapidly as possible connect the outlet tubes of the special weighing bottles (3 and 4) to the vacuum line and also connect each separately as shown in the Veitch-Jarrell diagram, to which reference has been made, to a train of two gas wash bottles containing fresh concentrated sulfuric acid and during the entire periods of drying allow a current of dry air to be *slowly* drawn (1 bubble per second) through the bottles containing the sample. (Some types of ovens have a ventilating hole on either side. The tubes connecting the bottles with the vacuum and sulfuric acid may be led to the outside through these openings.)

At the end of each 5 hour drying period and before removing the bottles from the oven, place the covers on the regular weighing bottles immediately upon opening the door of the oven. Then cut off the current of air from the special bottles and disconnect them from the vacuum line and the sulfuric acid, and at the same time place the ground-glass caps on the outlets of the cover. Remove all bottles containing the dried samples to tight desiccators containing fresh sulfuric acid, allow to cool for exactly 30 minutes, and weigh immediately. Calculate the percentage loss in weight as moisture after each 5 hour drying period.

After the drying of Sample No. 1 has been completed, weigh out the charges and determine moisture in the same way in Sample No. 2.

TOLUENE DISTILLATION METHOD.

(Charges 5 and 6.)

Immediately after connecting the flasks containing Charges 5 and 6 fill the receiving tube with toluene by pouring it through the top of the condenser. Carefully bring to a boil and distil at a rate of about 4 drops per second for exactly 2 hours. At the end of 1, 1¼, 1½, 1¾, and 2 hours' distillation, wash down the condenser by pouring toluene in at the top and at the same time brush thoroughly with a good quality tube brush attached to a stout copper wire and that has previously been boiled in toluene. Disconnect the receiving tube and dislodge any drops of water adhering to the sides by rubbing vigorously with a thin copper wire twisted at one end into a loop. Allow [illegible] tube to come to room temperature and read the volume of water collected very [illegible]fully to within 0.01 of a cc. Calculate the percentage of water in the sample. [illegible] to apply any necessary correction when calculating the results.)

When all work by both methods on Sample No. 1 has been completed, weigh out the charges and determine moisture in the same way in Sample No. 2.

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[1] *J. Am. Leather Chem. Assoc*, 1925, 20: 335.

[2] *This Journal*, 1925, 8: 296.

NOTES AND PRECAUTIONS.

Oven drying method.—The temperature within the oven must be frequently watched and carefully maintained at exactly 100°C. It must be read from a standardized thermometer located at such a position that the end of the mercury bulb rests very close to the bottles containing the sample. If this cannot be done carefully with all four bottles in the oven at the same time, complete the drying of Charges 1 and 3 only, and then dry Charges 2 and 4, and place them on the immediate left and right of the bulb of the thermometer.

Special care should be taken not to allow outside air of the drying and weighing rooms to come in contact with the contents of the bottles, which should be kept in the desiccators at all times between the periods of drying. The tops should always be kept on the bottles when out of the oven. All weighings should be done as rapidly as possible.

Toluene distillation method.—Carefully calibrate the tube before starting the work by adding, from an accurate Mohr's pipet, 2 cc. of water to 200 cc. of toluene in a 500 cc. Erlenmeyer flask and distil in the same manner as described previously. Read the receiving tube accurately within 0.01 of a cc. and mark the necessary correction on the tube. This correction, which *must* be applied to all regular determinations made by this method, not only applies to any incorrect graduations of the tube but it takes into account any error of manipulation. It is advisable to make several blank determinations on each tube in this way, using a definite quantity of water in the toluene before starting the work on the samples in order to familiarize yourself with the working details and also to make sure the correction is accurate (the usual correction ranges from +0.03 to +0.06 cc.). The condenser and receiving tube *must be clean* to prevent, so far as possible, water adhering to the sides of the glass. Before starting the work allow the tubes and condensers to remain in chromic-sulfuric acid overnight, rinse with distilled water and finally with alcohol, and dry either in an oven or by passing a current of air through them. This is usually necessary before each distillation.

Special care must be taken to see that any water that may stick to the sides of the condenser and tube during distillation has been washed and brushed down to the top of the water column in the tube.

The toluene *must* boil between 110°C. and 112°C. at 760 mm. pressure.

Always read the bottom of the meniscus when standardizing the tubes and also when making determinations.

COMMENTS BY COLLABORATORS.

L. A. Cuthbert: The moisture determinations specifying the regular weighing bottles were made in a new Weber electric drying oven. The temperature was controlled to within one-half a degree of 100°C. for practically the entire 5 hour period. There was a steady loss of weight during the 25 hours' drying by this method. The weather changed considerably during the five 5-hour periods, ranging from a bright clear day [illegible] a very rainy day.

The size of the weighing bottle does not appear to affect this method appreciably. While running Sample No. 2, I used one weighing bottle 45 mm. wide by 80 mm. high with 5.0 grams of leather, and another bottle 30 mm. wide by 50 mm. high, with 2.5 [illegible] of leather.

[illegible] determinations made by the toluene distillation method offered no difficulties. [illegible] it essential to have the equipment absolutely clean. Even with thorough [illegible], however, some small particles of water adhered to the sides of the tube just beneath the surface of the toluene and could not be removed with a copper wire. If these tubes are used, I would suggest putting a straight glass stopcock on the lower end of the calibrated part of the tube. The tube could be accurately graduated to 3 or 4 cc. and before starting a determination the lower part could be filled with water to the zero graduation.

V. J. Mlejnek and K. H Knight: The regular weighing bottles used were 50 mm. wide, 105 mm. high. Combined evaporator and dryer was used with maximum plate temperature of 98°C. As the house vacuum line was inconvenient, the vacuum was supplied by water displacement; therefore accurate control of a bubble per second was not possible. During the first week, with Sample No. 1 and using the special weighing bottles, the duplicates were connected in series with one set of two wash bottles. The order was reversed for each 5-hour drying period, and it was noted that for each period the leather that received the dry air gave the greater percentage of moisture. With Sample No. 2 each bottle had its own series of wash bottles, and uniform results were obtained.

Our regular method was tried (5 grams dried for 16 hours in open tannin dishes): Sample No. 1, 10.32 per cent; No. 2, 7.84 per cent.

The greatest difficulty with the distillation method is to get the Bidwell and Sterling tubes clean; thorough cleaning with soap and water and then 3 days' contact with chromic-sulfuric acid gave only fairly satisfactory readings of menisci. The method is quick in actual execution, but requires considerable attention and results are possibly only accurate to 0.05 per cent.

A. C. Orthmann.—I believe the toluene distillation method for the determination of moisture in leather to be very practical and efficient. This method measures the water directly and I obtained better checks by using this method than by oven drying.

Our electric oven showed considerable variation in temperature, as much as 5° above and below 100°C.

R. E. Porter.—We did not have an electric or other oven with thermostat control so we used a small air, gas-heated oven which we found was extremely difficult to control owing to variations in gas pressure, etc.

The toluene distillation method worked nicely, although the last 0.05 cc. is hard to get out. This is very important in accurate work. The toluene used boiled at 112°C.

J. H. Mitchell.—I found it somewhat difficult to check the toluene distillation method.

DISCUSSION BY REFEREE.

Oven drying method.—Ten collaborators reported results on Sample No. 1 and eleven reported on Sample No. 2. The first six collaborators listed in the table under Sample No. 1 and the first seven listed under Sample No. 2 reported using electric air ovens maintained at exactly 100°C. Collaborators 8, 9, 10, and 11 reported using various kinds of ovens with temperatures varying from 95° to 105°C. By comparing the results obtained in electric air ovens at 100°C. with the regular weighing bottle, the extreme variation is about 0.65 per cent on Sample No. 1, and about 0.90 per cent on Sample No. 2. The extreme variation of results by Collaborators 8, 9, 10, and 11 using the same kind of weighing bottle but working at a temperature range of 95° to 105°C. is over 2.00 per cent on Sample No. 1 and over 1.00 per cent on Sample No. 2.

When the special weighing bottle with a current of dry air passing through the sample during drying was used in the electric oven at 100°C., the extreme variation was about 0.45 per cent on Sample No. 1 (omitting Collaborator 5 for the first 5-hour period), and over 1.00 per cent on Sample No. 2. When the temperature, however, ranged from 95° to

105°C., as with Collaborators 8, 9, and 10, the extreme variation was about 1.50 per cent on both samples.

The variations in results are quite typical of those generally obtained in collaborative work on the determination of moisture in organic materials by the usual oven-drying procedures. It is believed by the referee that the great variations reported are largely due to faults in the method and also to the failure of some collaborators to follow the directions exactly.

It has been brought to the attention of this association by a former referee[1] that when leather is dried in an air oven, an ordinary open weighing bottle or dish being used, an appreciable variation in results will be obtained because of differences in the relative humidity of the air inside the oven during the drying period. But when leather is dried in the same kind of oven and at the same temperature, a special weighing bottle being used[2], and dry air is passed through the sample during drying, the relative humidity of the surrounding atmosphere will not affect the results. This method in the hands of the referee always gave higher moisture results than when an open container was used. This, however, is not always true in the collaborative results here reported since several collaborators obtained lower results with the special bottle. The reason for this was not determined. A slight leakage of moist air into the weighing bottle during drying or the lack of free dry air passing through the sample might be offered as an explanation.

Toluene distillation method. All collaborators reported results by the toluene distillation method, and all are in good agreement except those of Collaborators 8 and 10 for Sample No. 1. If these two results, which are apparently low, are omitted, the maximum result on Sample No. 1 is 11.70 per cent and the minimum is 11.23 per cent, a difference of 0.47 per cent. The maximum result on Sample No. 2 is 8.36 per cent and the minimum is 7.81 per cent, a difference of 0.55 per cent. The results by the toluene distillation method show, on a whole, a better agreement among the different analysts than those obtained by oven drying.

Much work done by the referee on the toluene distillation method indicates that it gives results that agree very well with those obtained by heating for five hours in an electric air oven at 100°C. while passing dry air through the sample. They also agree fairly well with the results obtained when the regular weighing bottle is used and the sample is [illegible] for from 15 to 25 hours at 100°C., provided the relative humidity of the drying room is practically constant at about 30 per cent. If, however, the relative humidity in the drying room is much higher than 30 per cent, the results are lower when the regular bottle is used.

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[1] *This Journal*, 1921, **5** 32, 389, 1923, **6** 309

[2] *J Am Leather Chem Assoc*, 1925, **20** 335

Results of collaborative A. O. A. C. work on the determination of moisture in leather.
(Results expressed in percentage.)

COLLABORATOR	REGULAR WEIGHING BOTTLE*					SPECIAL WEIGHING BOTTLE* (Dry air passed through sample during drying)					TOLUENE DISTILLATION METHOD
	Hours of drying					Hours of drying					
	5	10	15	20	25	5	10	15	20	25	
	SAMPLE NO. 1—VEGETABLE TANNED SOLE LEATHER										
1	10.73	11.08	11.28	11.25	11 42	11.20	11.40	11.58	11.67	11.83	11.35
2	10 57	10.94	11.06	11.12	11.10	11 09	11.37	11.45	11.51	11.57	11.25
3	10.66	11.02	11.17	11.29	11.33						11.28
4	10.10	10.37	10.54	10.67	10.73	11.22	11 36	11 61	11.80	11.80	11.23
5	10.35	10.97	11.05	11.13	11.12	9.43	10 87	11.12	11.43	11.47	11.32
7	10.34	10.57	10.77	10.83	10.89						11.40
8	11.68	12.34	12.50	12.72	12.53	11.06	11 84	11.68	11.68	11.48	10.65
9	10.93	11.14	11.17	11.26	11.37	10.93	11 40	11.48	11 61	11.85	11.70
10	9.61	9.71	9.75	10.48	10.46	9 51	9.72	9.93	10.42	10.92	10.37
11	9.90	10.03	10.12	10.60	10.67		.			..	11.62
Highest	11.68	12.34	12.50	12.72	12.53	11.22	11.84	11.68	11.80	11.85	11.70
Lowest	9.61	9.71	9.75	10.48	10.46	9.43	9.72	9.93	10.42	10.92	10.37
Difference	2.07	2.63	2.75	2.24	2.07	1.79	2.12	1.75	1.38	0.93	1.33
	SAMPLE NO. 2—BLACK GREASED HARNESS LEATHER										
1	7.80	8.02	8.08	8.04	8.13	8.08	8 20	8.24	8.27	8.34	8.00
2	7 77	7.83	8.02	8.08	8.18	8.07	8.16	8.27	8.29	8.41	8.00
3	7.98	8.01	8.13	8 18	8.19	.		.	.	. . .	8.13
4	7.61	7.74	7.84	7.90	7.99	8.08	8.36	8.36	8.40	8.48	8.18
5	7.49		7.70	7.71	7.73	7.36		7.50	7.93	8.02	7.81
6	7.96	8.11	8.76	8.80	9.06	8 55	8 61	8.94	9.09	9.16	8.36
7	7.81	7.94	7.95	8.08	8 15				.	. .	8.30
8	8.18	8.43	8.90	9.24	9.29	7.77	7.94	8.02	8.16	8.14	8.00
9	7.98	7.85	7.93	7.91	7.94	8.06	8.04	8.16	8.28	8.28	8.30
10	7.38	7.41	7.44	7.72	7.72	5.61	6.11	6.67	7.45	7 73	7.82
11	7.20	7.80	7.90	7 90	7.97						8.30
Highest	8.18	8.43	8.90	9.24	9.29	8.55	8.61	8.94	9.09	9.16	8.36
Lowest	7.20	7 41	7.44	7.71	7.72	5.61	6.11	6.67	7.45	7.73	7.81
Difference	0 98	1.02	1.46	1.53	1 57	2.94	2.50	2.27	1.64	1.43	0.55

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* Dried in oven.
Collaborators 1 to 7, inclusive, reported using electric air oven at 100°C
Collaborator 8 reported using electric air oven varying from 95° to 105°C
Collaborators 9 and 10 reported using steam air oven varying from 98° to 99°C.
Collaborator 11 reported using gas air oven varying from 95° to 105°C
Collaborators 1 and 2 made all determinations in the same laboratory, at different times, and used the same equipment throughout but otherwise worked independently.

All collaborative work on the determination of moisture in leather has shown that drying in an oven around 100°C. cannot be depended upon to give consistent results in the hands of different analysts. Probably the variations in atmospheric humidity in the oven during the period of drying are chiefly responsible for the discordant results, but there are also variations in temperature. Some ovens cannot be automatically controlled within close temperature ranges, and some are not uniformly heated in different parts. The toluene distillation method, however, gives more consistent moisture results in the hands of different analysts than does the usual oven drying method, probably because the temperature is definitely and automatically controlled during the entire period of distillation, and also because the relative humidity of the surrounding atmosphere during the distillation will affect the results but little, if any.

RECOMMENDATIONS[1].

It is recommended—

(1) That the tentative method for the determination of moisture in vegetable tanned leather[2] be dropped.

(2) That the toluene distillation method for the determination of moisture in vegetable tanned leather be adopted as tentative. This method has been published[3].

REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. **Graham** (Insecticide and Fungicide Laboratory, Bureau of Chemistry, Washington, D. C.), *Referee.*

The work done this year on insecticides and fungicides related to the following subjects:

1. Methods for the determination of cyanogen and chlorine in sodium and potassium cyanides.
2. Methods for the determination of cyanogen and chlorine in calcium cyanide.
3. Methods for the determination of water in soap.
4. Methods for the complete analysis of mineral oil-soap emulsions.
5. Methods for the determination of unsulfonated residue in petroleum spray oils.

DETERMINATION OF CYANOGEN AND CHLORINE IN SODIUM AND POTASSIUM CYANIDES.

Following the suggestions of the referee in last year's report, preliminary experiments were made relating to the determination of cyanogen and chlorine in sodium and potassium cyanides, the official method of analysis being used. As the method for chlorine was found to be unsatisfactory, particularly in the presence of sulfides, it was decided to utilize for collaborative work the methods embodying the suggestions of firms handling these materials.

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**: 60.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 79.
[3] *This Journal*, 1925, **8**: 295; 1927, **10**: 31.

One sample submitted was sodium cyanide containing a small quantity of sulfides, and the other was potassium cyanide with which was mixed about 10 per cent of sodium chloride.

The methods follow:

CYANOGEN.

REAGENTS.

(a) *0.1 N silver nitrate solution.*—Standardize against pure sodium chloride by titration, using chromate indicator; or gravimetrically, weighing the chloride.

(b) *Lead carbonate.*

(c) *10 per cent sodium hydroxide solution.*

(d) *Potassium iodide.*—Crystals or a saturated solution.

DETERMINATION.

Method I. Official Method.

Proceed as directed in par. **103**[1].

Method II.

Break the sample into small lumps in a mortar (do not grind), quickly weigh about 5 grams in a weighing bottle, and wash it into a 500 cc. volumetric flask containing about 200 cc. of water. Add a little lead carbonate as a precaution to precipitate any sulfide sulfur that may be present. Make to volume with water, filter, and transfer a 50 cc. aliquot to a 400 cc. beaker. Add 200 cc. of water, 5 cc. of the sodium hydroxide solution, 10 drops of the potassium iodide solution (or a few crystals), and titrate to a faint opalescence with the 0.1 N silver nitrate solution. In making this titration it is advantageous to have the beaker over a dark background. From the number of cc. of 0.1 N silver nitrate used calculate the percentage of cyanogen in the sample, using the following equation:

$$2\ NaCN + AgNO_3 = NaCN\ AgCN + NaNO_3.$$

Method III.

Proceed as in Method II except to replace the 5 cc. of 10 per cent sodium hydroxide solution with 1 cc. of strong ammonia.

CHLORINE.

REAGENTS.

(a) *0.1 N silver nitrate solution.*—Standardize against pure sodium chloride by titration, using chromate indicator; or gravimetrically, weighing the chloride.

(b) *0.1 N ammonium or potassium thiocyanate solution.*

(c) *Formaldehyde solution.*—A 40 per cent solution free from chlorides.

(d) *Ferric indicator.*—A saturated solution of ferric ammonium alum.

DETERMINATION.

Method I.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a beaker and dilute with an equal quantity of water, add 1 to 2 cc. of a 40 per cent

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 65.

TABLE 1.

Collaborative results—sodium and potassium cyanides.

ANALYST	SODIUM CYANIDE						POTASSIUM CYANIDE				
	Cyanogen			Chlorine			Cyanogen			Chlorine	
	Method I (Official)	Method II	Method III	Official Method	Method I	Method II	Method I (Official)	Method II	Method III	Method I	Method II
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
R. S. Gifford, American Cyanamid Co., New York, N. Y.	.	48.12	.	.		0.56*	.	33.97	.		6.42*
	. .	48.12	.	. .	.	0.55*		33.98	.		6.46*
Average	.	48.12	. .	.	.	0.56	.	33.98	. .		6.44
J. J. T. Graham	48.45	48.02	48.02	0.84	0.40	0.49	33.93	33.93	33.98	6.36	6.18
	48.29	47.92	48.14	0.77			33.98	33.93	34.14	6.33	6.19
Average	48.37	47.97	48.08	0.81	0.40	0.49	33.96	33.93	34.06	6.35	6.19
C. E. Swift, California Cyanide Co., Los Angeles, Calif.	47.77	48.03	47.87	. . .	0.61	0.61	33.56	33.80	33.80	6.04	5.80
L. B. Smith, Roessler, Hasslacher Co., Perth Amboy, N. J.	48.28	48.32	48.26	. .	0.54	0.51	34.22	34.16	34.15	6.22	6.24
	48.33	48.27	48.29		0.51	0.50	34.16	34.12	34.13	6.49	6.48
	48.27	48.32	48.26	. .	0.52	0.48	34.15	34.16	34.13	6.56	6.34
	48.32	48.34	48.32	. .	0.50	0.49	34.16	34.15	34.13	6.50	6.45
Average	48.30	48.32	48.28		0.52	0.50	34.17	34.15	34.14	6.44	6.38
General Average	48.24	48.16	48.17	0.81	0.51	0.52	34.02	34.02	34.07	6.36	6.28
Average deviation from the mean	0.14	0.13	0.13	.	0.04	0.04	0.17	0.11	0.10	0.14	0.15

* Chlorine titration made by the Mohr method.

solution of formaldehyde, stir well, and allow to stand 15 minutes. Acidify with nitric acid (5 cc. of 1 + 1 is usually enough) and 0.1 *N* silver nitrate in excess, stir well, filter, wash, and titrate the excess silver with 0.1 *N* thiocyanate solution, using ferric indicator. From the number of cc. of 0.1 *N* silver nitrate solution used, less the number of cc. of 0.1 *N* thiocyanate solution, calculate the percentage of chlorine in the sample.

Method II.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a distilling flask, dilute to 100–150 cc., acidify with a slight excess of acetic acid and distil, passing the vapors through a condenser whose delivery end dips into a solution of sodium hydroxide to absorb the hydrocyanic acid. After all the hydrocyanic acid is driven off, which should occur in the first 50 cc. of the distillate, wash the liquid from the distilling flask into a beaker, add 5 cc. of dilute nitric acid (1 + 1) and an excess of 0.1 *N* silver nitrate solution, stir well, filter, wash, and titrate the excess silver in the filtrate with 0.1 *N* thiocyanate solution, using ferric indicator. From the number of cc. of 0.1 *N* silver nitrate solution used, less the number of cc. of 0.1 *N* thiocyanate solution, calculate the percentage of chlorine in the sample.

The collaborative results are given in Table 1.

DISCUSSION OF RESULTS (TABLE 1).

Although in the official method for cyanogen there is no provision for the removal of sulfides, which are occasionally present in cyanide samples, the results by this method agree well with those obtained by Methods II and III. The end point, however, is somewhat vague in the presence of sulfides. In Methods II and III, in addition to the removal of sulfides with lead carbonate, the end point is made more distinct by the addition of potassium iodide. Under adverse conditions Method II will give better results than the present official method, of which it is a modification, and it is recommended, therefore, that this method be adopted in place of the present official method.

In the official method for chlorine any error in the end point of the cyanogen titration causes a corresponding error in the chlorine determination. In the case of a sample with a small chlorine content the relative error may be large. The use of potassium iodide as an indicator for the cyanogen determination as previously recommended would also interfere in the determination of chlorine by the official method. The referee recommends that both Method I and Method II be adopted as official methods for the determination of chlorine in place of the present official method.

DETERMINATION OF CYANOGEN AND CHLORINE IN CALCIUM CYANIDE.

One sample of commercial crude calcium cyanide was sent to the collaborators with the following directions:

CALCIUM CYANIDE.

CYANOGEN.

REAGENTS.

(a) *0.1 N silver nitrate solution.*—Standardize against pure sodium chloride by titration, using chromate indicator; or gravimetrically, weighing the chloride. 1 cc. of this solution is equivalent to 0.005202 gram of cyanogen. Factor CN to $Ca(CN)_2$ = 1.7702.

(b) *Soda-lead reagent.*—Dissolve 20 grams of lead acetate in 1 liter of water and add 200 grams of sodium carbonate, free from chlorides.

(c) *Sodium hydroxide solution.*—Dissolve 100 grams of sodium hydroxide in 1 liter of water.

(d) *Potassium iodide.*—Crystals, or a saturated solution.

DETERMINATION.

Method I.

Put about 200 cc. of water in a 500 cc. volumetric flask and carefully dry the neck. Weigh about 5 grams of the sample in a weighing bottle and transfer it to the flask with the least possible exposure to the air. Wash the sample down into the flask and mix by whirling until solution is complete and the small quantity of calcium carbide has been decomposed. Then add 25 cc. of the soda-lead reagent or sufficient to remove sulfides, close the flask with a rubber stopper, and shake thoroughly, preferably for one-half hour. Make to volume, mix, and filter through a dry filter. Transfer a 50 cc. aliquot to a 400 cc. beaker and add 200 cc. of water, 5 cc. of the 10 per cent sodium hydroxide solution, and 10 drops of the potassium iodide solution (or a few crystals). Titrate with the silver nitrate solution to a faint permanent opalescence. In making this titration it is advantageous to have the beaker over a dark background.

From the number of cc. of standard silver nitrate solution used, calculate the percentage of cyanogen in the sample, using the following equation:

$$2\ NaCN + AgNO_3 = NaCN.AgCN + NaNO_3.$$

CHLORINE.

REAGENTS.

(a) *0.1 N. silver nitrate solution.*—Standardize against pure sodium chloride by titration, using chromate indicator; or gravimetrically, weighing the chloride.

(b) *0.1 N ammonium or potassium thiocyanate solution.*

(c) *Formaldehyde solution.*—A 40 per cent solution free from chlorides.

(d) *Ferric indicator.*—A saturated solution of ferric ammonium alum.

DETERMINATION.

Method I.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a beaker and dilute with an equal quantity of water, add 1 to 2 cc. of a 40 per cent solution of formaldehyde, stir well, and allow to stand 15 minutes. Acidify with nitric acid (5 cc. of 1 + 1 is usually enough), add 0.1 *N* silver nitrate in excess, stir well, filter, wash, and titrate the excess silver with 0.1 *N* thiocyanate solution, using ferric indicator. From the number of cc. of 0.1 *N* silver nitrate solution used, less the number of cc. of 0.1 *N* thiocyanate solution, calculate the percentage of chlorine in the sample.

Method II.

Transfer a 50 cc. aliquot of the solution prepared for the determination of cyanogen to a distilling flask, dilute to 100–150 cc., acidify with a slight excess of acetic acid, and distil, passing the vapors through a condenser whose delivery end dips into a solution of sodium hydroxide to absorb the hydrocyanic acid. After all the hydrocyanic acid is driven off, which should occur in the first 50 cc. of the distillate, wash the liquid from the distilling flask into a beaker, add 5 cc. of dilute nitric acid (1 + 1) and an excess of 0.1 *N* silver nitrate solution, stir well, filter, wash, and titrate the excess silver in the filtrate with 0.1 *N* thiocyanate solution, using ferric indicator. From the number of cc. of 0.1 *N* silver nitrate solution used, less the number of cc. of 0.1 *N* thiocyanate solution, calculate the percentage of chlorine in the sample.

The collaborative results are given in Table 2.

TABLE 2.

Collaborative results—calcium cyanide.

ANALYST	CYANOGEN	CHLORINE	
		Method I	Method II
	per cent	*per cent*	*per cent*
R. S. Gifford	25.44 25.45	19.54* 19.49*	
Average	25.45	19.52	
J. J. T. Graham	25.53 25.52	19.30 19.30	19.30 19.26
Average	25.53	19.30	19.28
C. E. Swift	25.4	18.8	19.0
General Average	25.47	19.29	19.19
Average deviation from the mean	0.05	0.19	0.12

* Chlorine titration made by the Mohr method.

The results for cyanogen and chlorine in the sample of calcium cyanide are so satisfactory that the referee considers these methods to be worthy of adoption as official methods.

DETERMINATION OF WATER IN SOAP.

Samples of fish oil soap similar to those described in the 1925 report[1] were prepared. Samples 1 and 2 were potash and soda soaps, respectively. These were packed in glass jars and sent to the collaborators with the following methods:

Xylene Distillation Method.

Weigh about 20 grams of the sample into a 300–500 cc. flask; add 50 cc. of xylene; and, in order to prevent foaming, add about 10 grams of lump rosin. Do not use powdered rosin because it usually contains an appreciable quantity of moisture. Distil into a Dean and Stark type distilling tube receiver[2], and continue the distillation until no more water collects in the receiver. Allow the contents of the tube to cool to room temperature, read the volume of water under the xylene in the tube, and from this volume calculate the percentage of water in the sample.

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[1] *This Journal*, 1926, **9**: 134.
[2] *J. Ind. Eng. Chem.*, 1920, **12**: 486.

Official Method.

Proceed as described in par. **105**[1], or par. **61**[2].

The collaborative results are given in Table 3.

TABLE 3.

Collaborative results—water in soaps.

ANALYST	SAMPLE I		SAMPLE II	
	Official Method	Xylene Distillation Method	Official Method	Xylene Distillation Method
	per cent	*per cent*	*per cent*	*per cent*
W. A. De Long	22.3	23.7	20.6	22.1
Macdonald College, Canada	22.8	24.0	20.8	22.5
Average	22.5	23.9	20.7	22.3
J. W. Elmore	23.3	23.5	20.9	22.5
Department of Agriculture	23.2	23.5	21.0	22.5
Sacramento, Calif.				
Average	23.3	23.5	21.0	22.5
H. J. Fisher	23.4	23.4	20.7	22.7
Agricultural Experiment Station	22.7	23.7	20.5	23.5
New Haven, Conn.				
Average	23.0	23.5	20.6	23.1
J. J. T. Graham	22.5	24.0	22.5	22.2
	22.7	23.8	21.8	22.3
Average	22.6	23.9	22.1	22.3
General average	22.9	23.7	21.1	22.5
Average deviation from the mean	0.3	0.2	0.5	0.3

DISCUSSION OF RESULTS (TABLE 3).

The results by the xylene distillation method are quite uniform. A greater variation exists among the results obtained by the official drying method than among those by the xylene distillation method, and the results by the latter method are about 1 per cent higher. This is probably due to the fact that when the official method was used, the partial oxidation of the fatty acids of the soap during the drying caused the results for moisture to be correspondingly low.

One of the collaborators comments on this method as follows: "In determining water in Sample 2 by the official method the minimum weight stage was found to be of brief duration, the decrease due to loss of water being closely followed by a rapid rise, presumably due to oxidation. Thus in one determination the sample lost 1.16 per cent of its weight in a 30 minute heating period and gained 1.73 per cent during the next period (in terms of percentage of the minimum weight recorded). Accordingly, for soaps of this type, the estimation of moisture content from loss of weight under the conditions of the method can be but a rough approximation at best".

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 65.
[2] *Ibid.*, 1920, 64.

ANALYSIS OF MINERAL OIL—SOAP EMULSIONS.

Samples of kerosene and engine oil emulsions were prepared as described in last year's report[1], potash fish oil soap being used as the emulsifier. The emulsions were of good quality and showed no tendency toward separation of oil. They were sent to the collaborating chemists with the following directions:

WATER.

Weigh about 25 grams of the sample into a 300–500 cc. flask and add 50 cc. of xylene; if necessary to prevent foaming, add a small piece of rosin. Distil into a Dean and Stark type distilling tube receiver[2] and continue the distillation until no more water collects in the receiver. Allow the contents of the tube to cool to room temperature, read the volume of water under the xylene in the tube, and from this volume calculate the percentage of water in the sample.

TOTAL OIL.

(Modification of method for the determination of kerosene in kerosene emulsions[3]).

Weigh about 10 grams of the sample into a Babcock cream bottle. Dilute with about 10 cc. of hot water and add 5–10 cc. of dilute sulfuric acid (1 + 1).

Set the bottle in a hot water bath for about 5 minutes to hasten the separation of the oil, add sufficient saturated sodium chloride solution to bring the oil layer within the graduations on the neck of the bottle, whirl at a rate of 1200 revolutions per minute for about 2 minutes, and allow to cool. Read the volume of the oily layer, determine its density, and from these values calculate its weight and percentage. To obtain the percentage of oil in the sample, deduct from this percentage value the percentage of fatty acids (and phenols if present), determined separately.

SOAP.

Method I.

Evaporate about 10 grams of the sample in a platinum dish, ignite and leach the charred mass with water, and filter. Ignite the residue, take up with water, filter, add the filtrate to the leachings, and titrate with 0.1 *N* hydrochloric acid, using methyl orange as indicator. From the number of cc. of hydrochloric acid used, calculate the percentage of sodium or potassium oleate or rosin soap, according to which is present in the sample.

Method II.

Weigh 20 grams of the sample into a separatory funnel, add 60 cc. of petroleum ether, and extract the mixture once with 20 cc. and four times with 10 cc. of 50 per cent alcohol. Break the emulsion if necessary with 1 or 2 cc. of a strong solution of sodium hydroxide, allowing the solution to run down the side of the separatory funnel, which is then gently twirled and allowed to stand for a few minutes. Draw off the alcoholic layers and wash them successively through petroleum ether contained in two other separatory funnels. Combine the alcoholic extracts in a beaker and evaporate on a steam bath to remove alcohol. Dissolve the residue in about 100 cc. of water made alkaline with sodium hydroxide. Transfer to a separatory funnel, acidify with hydrochloric or sulfuric acid, extract three times with ether, and successively wash the ether extracts twice with water. Combine the ether extracts, evaporate in a weighed beaker on a steam bath, and weigh as fatty acids. From the weight of fatty acids calculate the percentage of soap in the sample as sodium or potassium oleate.

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[1] *This Journal*, 1926, 9: 128.
[2] *J. Ind. Eng. Chem.*, 1920, 12: 486.
[3] U. S. Dept. Agri. Bur. Chem. Bull 105, p. 165.

UNSULFONATED RESIDUE.

Method I.

REAGENT.

38 N Sulfuric Acid.—Prepare as directed under turpentine[1].

DETERMINATION.

Place 20 cc. of the 38 *N* sulfuric acid in a Babcock cream bottle. Add slowly from a pipet 5 cc. of the recovered oil, gently shaking or rotating the bottle and being careful that the temperature does not rise above 60°C. (After preliminary draining, in the case of heavy oils, warm the pipet by drawing it several times through the flame of a Bunsen burner and then drain thoroughly.) When the mixture no longer develops heat on shaking, agitate thoroughly by vigorously shaking for about one-half minute. Place the bottle in a water bath and heat at 60°–65°C. for 10 minutes, keeping the contents of the bottle thoroughly mixed by shaking vigorously for 20 seconds at 2 minute intervals during the heating period. Fill the bottle with strong sulfuric acid until the oil rises into the graduated neck. Centrifugalize for 5 minutes (or longer if necessary to obtain a constant volume of the oil) at 1200–1500 revolutions per minute. Read the volume of unsulfonated residue from the graduations on the neck of the bottle, and from this value calculate the percentage by volume of the unsulfonated oil.

Method II[2].

Determine the specific gravity of the oil at 25°C. and weigh the equivalent of 5 cc. into a Babcock cream bottle. Add slowly 20 cc. of exactly 37 *N* sulfuric acid in four equal portions, shaking after each addition and *taking care that the temperature of the mixture does not rise above 60°C.*, cooling in ice water if necessary. When the mixture no longer warms on shaking, agitate thoroughly, immerse the bottle in a water bath at 60°–65°C. and hold at this temperature for 1 hour, shaking every 10 minutes. Fill the flask with strong sulfuric acid (approximately 1.84 specific gravity) until the oil rises into the graduated neck, centrifuge to constant volume of the oil at approximately 1500 revolutions per minute, cool to 25°C., read the volume of the unsulfonated residue from the graduations on the neck of the flask, and calculate the percentage by volume.

ASH.

Evaporate 10 grams of the sample, or more if necessary, in a platinum dish; ignite and leach the charred mass with water. Ignite the residue, add the leachings, evaporate to dryness, ignite, and weigh. From this weight calculate the percentage of ash. Test the ash for copper, calcium, calcium fluoride, etc.

The collaborative results are given in Table 4.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 408.

[2] Method suggested to the referee by J. B. Terry, Chief Chemist of the Standard Oil Company of California.

TABLE 4.

Collaborative results—mineral oil-soap emulsions.

(Expressed in percentage.)

ANALYST	SAMPLE I							SAMPLE II						
	Water	Total oil	Soap		Unsulfonated residue*		Ash	Water	Total oil	Soap		Unsulfonated residue*		Ash
			Method I	Method II	Method I	Method II				Method I	Method II	Method I	Method II	
W. A. De Long	28.2	66.6	5.32	5.32	58.0	60.0	1.30	31.0	68.0	1.70	1.62	78.0	78.0	0.39
	28.5	66.5	5.48	5.23	58.0	58.0	1.29	31.3	68.5	1.68	1.41	78.0	76.0	0.42
Average	28.4	66.6	5.40	5.28	58.0	59.0	1.30	31.1	68.3	1.69	1.52	78.0	77.0	0.41
J. W. Elmore	28.1	65.2	5.40	5.56	57.6	62.0	1.23	30.8	66.2	1.69	1.79	72.8	78.0	0.40
	28.3	65.3	5.43	5.65	58.0	62.0	1.24	30.9	66.4	1.71	1.79	72.8	78.4	0.41
Average	28.2	65.3	5.42	5.61	57.8	62 0	1.24	30.9	66.3	1.70	1.79	72.8	78.2	0.41
H. J. Fisher	28.2	66.0	5.13	5.34	57.9	63.1	1.36	30.2	66.6	1.50	1.64	81.5	81.3	0.45
	28.2	65.6	5.32	5.38	57.5	64.3	1.31	30.3	67.8	1.58	1.63	82.3	84.7	0.45
Average	28.2	65.8	5.23	5.36	57.7	63.7	1.34	30 3	67.2	1.54	1.64	81.9	83.0	0.45
J. J. T. Graham	28.0	65.1	5.34	5.74	62.0		1.25	30.7	66.6	1.72	1.76	81.6	.	0.41
	28.0	65.0	5.37	5 76	62.4		1.26	31.3	66 9	1.73	1.60	83.2		0.42
Average	28 0	65.1	5.36	5.75	62.2		1.26	31.0	66.8	1.73	1.68	82.4		0.42
General Average	28.2	65.7	5.35	5 50	58.9	61.6	1.28	30.8	67 1	1.66	1.66	78 8	79.4	0.42
Average deviation from the mean	0.1	0.5	0.07	0.18	1.6	1.7	0 03	0.3	0.6	0.06	0.10	1.9	2.4	0.02

* Determinations were made on the recovered oil containing the fatty acids from the soap emulsifier

DISCUSSION OF RESULTS (TABLE 4).

An examination of Table 4 shows that there is close agreement among the results for water and ash. The methods for soap are in close agreement, but they are subject to certain inherent errors. In Method I the soap is calculated from the determination of total alkali, and in soaps containing a large excess of alkali error will result. In both methods error will result if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid. The latter objection is not serious because the molar weight of the fatty acids of soaps used in emulsions will not be likely to vary much from that of oleic acid. Method I is a rapid method and will be useful in many cases, but it is hardly worthy of adoption as an official method. Method II, with a footnote warning of the possible error due to variations in the molar weight from that of oleic acid, is of sufficient merit to be adopted as an official method.

The accuracy of the oil determination depends upon the accuracy of reading the Babcock bottles. The error in this reading may amount to 1 or 2 per cent, but when it is taken into account the variations in the results are not excessive.

The two methods for unsulfonated residue give equally uniform results. These and two other sulfonation methods were later sent out with three samples of oil for separate study. The results are reported in the following section.

ADDITIONAL WORK ON THE UNSULFONATED RESIDUE IN MINERAL OILS.

After the oil emulsion samples had been sent to the collaborators the referee was requested to make a further study of sulfonation methods for spray oils. Three samples of mineral oils widely used for spray purposes were secured and sent to the collaborators with the following directions:

UNSULFONATED RESIDUE IN MINERAL OILS.

REAGENTS.

38 N sulfuric acid.—Prepare as directed under turpentine.

37 N sulfuric acid.—Prepare 37 *N* acid in the same manner as directed above for 38 *N* acid. The standardized 37 *N* acid should contain 98.61 per cent H_2SO_4.

DETERMINATION.

Method I.

Determine the density of the oil at 25°C. and weigh the equivalent of 5 cc. into a Babcock cream bottle. The bottle should be about 15 cm. long and may be either the 9 gram 50 per cent or the 18 gram 30 per cent cream bottle. Add slowly 20 cc. of the 38 *N* fuming sulfuric acid, gently shaking or rotating the bottle, being careful that the temperature does not rise above 60°C. and cooling in ice water if necessary. When the mixture no longer develops heat on shaking, agitate thoroughly, place the bottle in a water bath, and heat at 60°–65°C. for 10 minutes, keeping the contents of the bottle thoroughly mixed by shaking vigorously for a period of 20 seconds at 2 minute intervals. Remove from the bath and fill the bottle with strong sulfuric acid until the oil rises into the graduated neck. Centrifugalize for 5 minutes (or longer if necessary to obtain a constant volume of the oil) at 1200–1500 revolutions per minute. Read the volume of unsulfonated residue from the graduations on the neck of the bottle, and from this value calculate the percentage by volume of the unsulfonated oil

Method II.

Same as Method II given previously in this report under Unsulfonated Residue. Methods II and IV were suggested to the referee by J. B. Terry.

Method III.

Proceed as in Method II, except to heat the bottles for 18 minutes and shake for 20 seconds at 3 minute intervals.

Method IV.

Proceed as in Method II, except to heat the bottles at a temperature of 100°C.

TABLE 5.

Physical characteristics of the oils.

CHARACTERISTIC	SAMPLE 1	SAMPLE 2	SAMPLE 3
Specific gravity	0.878	0.927	0.905
Color	White	Red	Red
Flash	315°F.	320°F.	400°F.
Viscosity at 100°F. (Saybolt)	107 sec.	140 sec.	235 sec.
Pour	0	0	0

The collaborative results are given in Table 6.

DISCUSSION OF RESULTS (TABLE 6).

An analysis of the figures in Table 6 shows that the individual analysts obtained results that in nearly every case checked quite well on the same method and that when the nature of the determination is considered a majority of the analysts checked each other fairly well.

In the report of his collaborative work, W. A. De Long comments as follows:

> Methods I and III cause little or no charring of the sample, whereas Method II causes extensive carbonization and Method IV still more than does Method II. This effect was so marked in the case of both of the latter methods as to render accurate reading of the volume of unsulfonated residue impossible without resorting to the expedient of adding a few drops of water as recommended by the referee.
>
> The greater percentage of unsulfonated residue shown by Method IV on Sample 3 over that indicated by the other methods, is believed to be due to regeneration of the hydrocarbons as a result of the more extended period of heating at the higher temperature employed. Hence this method would not appear to be dependable for the estimation of the unsulfonated residue in oils of the type of Sample 3.
>
> The writer can see no advantages in the use of Methods II, III, or IV rather than Method I, so far at least as the samples of spray oils examined are concerned. On the other hand there is the already mentioned inaccuracy of Method IV when applied to

TABLE 6.

Collaborative results—unsulfonated residue in mineral oil.
(Expressed in percentage.)

ANALYST	SAMPLE I				SAMPLE II				SAMPLE III			
	Method I	Method II	Method III	Method IV	Method I	Method II	Method III	Method IV	Method I	Method II	Method III	Method IV
W. A. De Long	96.0 96.0	96.0 96.0	96.0 96.0	96.0 96.0	56.0 56.0	58.0 58.0	56.0 56.0	56.0 56.0	64.0 64.0	64.0* 64.0*	64.0 64.0	72.0* 72.0*
Average	96.0	96.0	96.0	96.0	56.0	58.0	56.0	56.0	64.0	64.0	64.0	72.0
J. W. Elmore	95.2 95.2	100.0 100.0	100.0 100.0	100.0 100.0	53.2 53.2	56.0 56.8	56.0 56. 4	58.0 58.0	63.6 64.0	66.0 66.4	66.0 66.0	70.4 70.8
Average	95.2	100.0	100.0	100.0	53.2	56.4	56.2	58.0	63.8	66.2	66.0	70.6
H. J. Fisher	99.9 101.9 98.0	102.3 101.9 . . .	101.3 102.3 .	100.9 102.3 . .	52.7 54.1 54.0	60.3 61.5 .	58.9 59.1	62.9* 66.9* .	67.1* 66.9* 66.4	73.7* 75.3* . . .	69.3* 68.7*	74.7* 74.3* . .
Average	99.9	102.1	101.8	101.6	53.6	60.9	59.0	64.9	66.8	74.5	69.0	74.5
J. J. T. Graham	96.0 96.0 . . .	99.0 99.6 99.6	99.2 99.6 . . .	100.4 100.4 . .	51.6 52.0 51.6	56.0 56.0 55.6	56.4 56.0	56.8 56.8 . .	63.6 64.0 . . .	72.8* 72.0* . . .	64.4 64.0	72.0* 71.6* . . . .
Average	96.0	99.4	99.4	100.4	51.7	55.9	56.2	56.8	63.8	72.4	64.2	71.8
E. L. Griffin Bureau of Chemistry Washington, D. C.	96.8 96.8	100.0 100.4	100.0 100.0	100.4 100.4	53.2 53.6	60.4 60.4	60.0 60.0	60.8 60.0	65.2 64.4	73.2* 72.8*	63.6 63.6	72.0* 72.8*
Average	96.8	100.2	100.0	100.4	53.4	60.4	60.0	60.4	64.8	73.0	63.6	72.4
J. B. Terry Standard Oil Co. of California Richmond, Calif.	95.0	99.0	99.0	98.0	51.0	53.0	55.0	56.0	60.0	73.0	64.0	71.0
General Average	96.9	99.5	99.4	99.5	53.3	57.7	57.3	58.9	64.4	70.3	65.2	72.1
Average deviation from the mean	1.52	1.32	1.35	1.57	1.16	2.10	1.65	2.70	1.13	3.80	1.64	0.95

* "Carbonization" of the oil reported by analyst

oils of the character of Sample 3, and also the greater length of time required for the operation of both this method and Method IV to be considered

A very good discussion of these methods is also given by E. L. Griffin, who comments as follows:

> I prefer Method I for the following reasons:
>
> First: I believe the results mean as much as, or more than, the results obtained by the other methods. The results obtained on the paraffin or mixed base oil, No. 3, were the same by Methods I and III. The naphthene base oil, No. 2, gave a lower result by Method I. Whether this difference means anything in the way of actual injury to foliage would have to be shown by experiment.
>
> Second: The shorter time for the determination is an advantage.
>
> Third: The method is the same as that for unpolymerized residue in turpentine except for minor details and the one acid may be used in both cases. No serious difficulty has been found in making and keeping this acid.
>
> Using Methods II, III, and IV the same results were obtained on Oils 1 and 2. Method III was the best of these methods both because of the shorter time required and because of the smaller chance of accident at the lower temperature. In Method IV there is the possibility that the bottles will be shaken less than in the other methods because it is a little more difficult to handle them at the higher temperature.
>
> With Oil 3, Methods II and IV gave a black, tar-like residue, which could not be read except by the aid of a few drops of water to form a layer. The results were about 10 per cent higher than by either of the other methods, probably because of suspended colloidal tarry material formed by polymerization due to long heating in the presence of acid. Neither method was satisfactory.

Of the six analysts participating in this work, four expressed a strong preference for Method I and condemned Method IV as the least desirable of all the methods. On the other hand the two other analysts seemed to think that the best results were obtained by Method IV. Four of the analysts reported trouble with Methods II and IV owing to carbonization of the oil with Sample 3, and while the two other analysts did not report this difficulty their results were considerably higher by these two methods. This "carbonization" seems to occur in the sulfonation of paraffine base oils of high viscosity. Usually the naphthene base oils do not react in this way and a clear oil results, even in the case of oils having a viscosity as high as 300 seconds.

The condition occurring in the sulfonations by Methods II and IV, to which reference has previously been made under the name "carbonization", seems to be rather a formation of a tarry substance due to polymerization by the long heating of the oil in the presence of strong sulfuric acid. Only one analyst reported any carbonization by Methods I and III, in which the time of sulfonation was much shorter.

Gray and de Ong[1] state that charring is likely to occur if an acid of greater strength than 37 *N* is used in the sulfonation. The results of this work do not confirm their statement, but they point to the conclusion that the carbonization is caused by long heating rather than by strong acid.

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[1] *Ind. Eng. Chem.*, 1926, **18**: 177.

TEST OF THE ACCURACY OF MEASUREMENT OF THE OILS WITH A PIPET.

Since there was some doubt of the accuracy with which charges of heavy oil could be measured with a pipet, it was thought best in carrying out this work to weigh the charges of oil and to make a separate investigation of the accuracy with which the oil samples could be measured.

The following directions were sent to the collaborators along with the oils for the sulfonation tests:

Use a pipet calibrated at 20°C. to deliver 5 cc. Measure several portions of water at 20°C. into weighing bottles and determine the weight. The apparent weight of 1 cc. of water at 20°C. is 0.9972 gram. Calculate the volume of water delivered by the pipet.

Measure several portions of each of the oils at 20°C. in the following manner. After a preliminary draining, warm the pipet by drawing it several times through the flame of a Bunsen burner to reduce the viscosity of the oil, and then drain thoroughly. Since the viscosity of these oils is much greater than that of aqueous solutions, it is best to blow out the last drop of oil from the pipet. Determine the weight of the oil delivered and from this weight and the density of the oil, calculate the volume of the oil.

The collaborative results are given in Table 7.

TABLE 7.

Collaborative results—a comparison of the volume of oil and water delivered by a pipet at 20°C.

ANALYST	WATER	OIL			ERROR		
		Sample I	Sample II	Sample III	Sample I	Sample II	Sample III
		cc.	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
W. A. De Long	4.892	4.937 4.896 4 910	4 846 4.859 4.860	4 820 4.798 4.823			
Average	4 892	4 914	4.855	4.814	+0 45	−0.76	−1.59†
J. W. Elmore		5 021 5 025 5.019	5.027 5.032 5.017	5 010 5 003 5 016			
Average	4.982*	5.022	5 025	5 010	+0.80	+0.86	+0.56
H. J. Fisher	4.964	5.087	5.112.	4 954	+2.48	+2.98	−0 20
J. J. T. Graham	4.998	4.951	4.951	4 960	−0.94	−0.94	−0.76
E. L. Griffin	5.00	4 96 4.99	5.00 4.98	4.97 4.98			
Average	5 00	4.98	4.99	4.98	−0.40	−0.20	−0.40

* Average of seven determinations.

† By further heating and draining this error was reduced to −0.94 per cent.

The errors of measurement, as shown by the results in Table 7, are nearly all less than 1.0 per cent. This is well within the limit of accuracy of the sulfonation methods and proves that measuring the charges of oil will give practically as reliable results as weighing.

RECOMMENDATIONS[1].

It is recommended—

(1) That the methods for the determination of cyanogen and chlorine in sodium and potassium cyanides[2] be dropped as official methods.

(2) That Method II for the determination of cyanogen in sodium and potassium cyanides, as given in this report and published previously[3], be adopted as an official method.

(3) That Methods I and II for the determination of chlorine in sodium and potassium cyanides, as given in this report and published previously[4], be adopted as official methods.

(4) That Method I for the determination of cyanogen in calcium cyanide, as given in this report and published previously[5], be adopted as an official method.

(5) That Methods I and II for the determination of chlorine in calcium cyanide, as given in this report and published previously[5], be adopted as official methods.

(6) That the method for the determination of moisture in soap[6] be dropped as an official method.

(7) That the xylene distillation method for the determination of water in soap, as given in this report and published previously[7], be adopted as an official method.

(8) That the methods for the determination of water, total oil, and ash in mineral oil-soap emulsions, as given in this report and published previously[8], be adopted as official methods.

(9) That no further study be made of Method I for the determination of soap in mineral oil-soap emulsions, as given in this report, and that this method be dropped as a tentative method.

(10) That Method II for the determination of soap in mineral oil-soap emulsions, as given in this report and published previously[9], be adopted as an official method with the following note appended: "Error will result in this method if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid".

(11) That in Method I for the determination of unsulfonated residue in mineral oils, given in this report, the following be substituted for the

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**. 61.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 65.
[3] *This Journal* 1927, **10**: 27.
[4] *Ibid.*, 28.
[5] *Ibid.*, 29.
[6] *Methods of Analysis, A. O. A. C*, 1925, 65.
[7] *This Journal*, 1926, **9**: 28.
[8] *Ibid.*, 28, 29.
[9] *This Journal*, 1926, **9**: 28.

first sentence and that the method then be adopted as an official method for the determination of unsulfonated residue in mineral oils and the recovered oil obtained in the analysis of oil-soap emulsions.

With a pipet, measure 5 cc. of the oil into a Babcock cream bottle (after preliminary draining, in the case of heavy oils, warm the pipet by drawing it several times through the flame of a Bunsen burner to reduce the viscosity of the oil, and then drain thoroughly), or in lieu of the above procedure determine the density of the oil and weigh the equivalent of 5 cc.

This method, with the change recommended by the referee, has been published[1].

Recommendations 7, 8, 9, 10, and 11, with some revision, cover methods that were adopted as tentative methods at the 1925 meeting.

REPORT ON SOILS AND LIMING MATERIALS.

By W. H. MacIntire (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Referee.*

During the past year two subjects have been considered in the laboratories of the referee—the determination of manganese and some of the less common elements in soils and studies on the solubility of soil potassium. The manganese content of a number of soils was determined by the method as now given[2], and it appears that the results are often far below the true values as determined by oxidative colorimetric determinations upon original aliquots. The precipitation of iron, aluminum, and phosphorus was followed by treatment with ammonium persulfate, with subsequent redissolving, reprecipitation, and reoxidation, so that the ignited precipitates represented the total oxides of iron, aluminum, manganese, and phosphorus. From this total the directly determined iron, manganese, and phosphorus were substracted to give aluminum. In view of recent work, which has indicated that the occurrence of manganese is more important than has been thought, it is recommended that further study be given to the accuracy of the present procedure. If necessary, an associate referee should be appointed for the purpose of formulating a more accurate method.

In view of the more recent work upon the occurrence of the less common elements—such as arsenic, copper, tin, and zinc—in some soils, it appears desirable that a study of such occurrences and their determination be made. It is recommended, therefore, that the associate referee appointed to study manganese be requested to include such studies as a part of his efforts and be designated as Associate Referee on Manganese and the Less Abundant Elements.

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[1] *This Journal*, 1927, **10**: 30.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 30.

The solubility of soil potassium was studied by the following procedures:

(1) Digestion with hydrochloric acid, specific gravity 1.115, for 10 hours in a water bath, ratio 1 to 10;

(2) Digestion with 0.2 *N* hydrochloric acid at room temperature, ratio 1 to 5;

(3) Extraction to equilibrium with 0.05 *N* hydrochloric acid;

(4) Extraction to equilibrium with normal ammonium chloride;

(5) Digestion with distilled water at room temperature, ratio 1 to 5; and

(6) Digestion with distilled water saturated with carbon dioxide at room temperature and pressure, ratio 1 to 5.

These studies have been carried out with the thought that an acceptable method may be found for the determination of the availability of potassium. Investigations on the availability of calcium and phosphorus also seem to be timely in view of the foreign and American work upon interchange of bases and the work relating to "reciprocal repression" in this country. In so far as the potassium results are concerned numerous data are now at hand, but these are being verified prior to publication. It is recommended, therefore, that this work be continued and reported later by the referee.

The referee concurs in the recommendations of the Associate Referees, further recommending, however,

(1) That the recommendations of the Associate Referee on Reaction Value of Soils be formulated into definite directions by the Committee on Revision of Soil Analysis, and

(2) That determined reaction values be expressed in terms of "specific acidity" and "specific alkalinity", with corresponding exponential (pH) values given in brackets and that a table of corresponding values for the two methods be appended.

REPORT ON REACTION VALUE OF SOILS.

By P. S. Burgess (Agricultural Experiment Station, Tucson, Ariz.), *Associate Referee.*

As a result of the work done last year by the associate referee in his laboratory, and as a result of correspondence with others who had had considerable experience with the electrometric determination of H ions in soils, the following points would seem to be fairly well settled:

1. The best proportion of water to soil is 5 to 1. Greater dilutions have nothing to recommend them, while less water with certain muck and clay soils may give too thick a magma. Furthermore, the proportion of 5 to 1 is at present almost universally used.

2. The optimum length of time for shaking the suspensions before taking the readings appears to be approximately 30 minutes. Intermittent shaking is as good as constant agitation.

3. Whenever possible, fresh soils should be used, as even air drying has a tendency towards increased acidity. When air-dried or over-dried soils are employed, it should be so reported.

The work of the associate referee this past year has been confined to a study of the effect on electrometric pH determinations of (1) nitrates and (2) electrolytes ("white alkali" salts) in soils.

Without giving the many data that were obtained on an alkaline soil, it may be stated that last year's results—showing that nitrates will not interfere with the electrometric determination of H ions when present in concentrations below 400 parts of NO_3 per million parts of soil—have been largely corroborated. Concentrations greater than this are seldom found in arable soils. In the presence of larger quantities of nitrates the suspensions usually settle out readliy (at least when centrifuged), giving extracts of sufficient clarity for colorimetric work.

That neutral electrolytes, as the chlorides and sulfates of sodium, potassium, calcium, and magnesium, may have an effect on pH measurements has been shown by Arrhenius[1], Atkins[2], and Crowther[3]. In arid or semi-arid areas, the presence of these soluble salts ("white alkali") in the soils is very common, hence such effects are especially important to soil chemists in sections of the West and Southwest.

The accompanying table shows the results of added sodium chloride and sodium sulfate on the pH values of soils as determined electrometrically. It will be noted that the Kentucky soil was acid, the Oregon soil was approximately neutral, while the Arizona soil was a calcareous soil (over 4 per cent of calcium carbonate) and distinctly alkaline. None of these soils carried soluble sulfates or chlorides (the Arizona soil was washed free from traces of sulfates and chlorides and dried before use). The Kentucky soil belongs to the Waverly Series (silt loam) and came from Campbellsville; the Oregon soil was designated as Yakima sandy loam (high in organic matter) and came from Klamath Falls; while the Arizona sample was a sandy loam low in organic matter from near Tucson (not mapped by U. S. Bureau of Soils). The salts were added as parts per million of dry soil (see table), made up with CO_2-free distilled water in the proportion of 5 to 1 of soil, and agitated intermittently for approximately thirty minutes. The pH determinations were made on the electrode in the usual way.

It will be noted from the table that with both salts employed and with all three soils there was a slow progressive increase in acidity (or decrease in alkalinity) as the quantities of added salts were increased. In the case

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[1] Proc. Int. Soc. Soil Sci., 1925, in Intern. Rev. Sci. and Prac. Agri., New Series, Vol. 3, No. 1, p. 123.
[2] Agr. Research Inst., Pusa, India, Bull. 136, 1922.
[3] *J. Agr. Sci.*, 1925, 15: 210.

*Effect of NaCl and Na_2SO_4 on pH measurements**.

SALT ADDED (PARTS PER MILLION)	NaCl		Na_2SO_4	
	pH	Specific acidity	pH	Specific acidity
	KENTUCKY SOIL (ACID)			
0	6.17	6.8	6.17	6.8
1000	6.15	7.1	6.15	7.1
5000	5.90	12.5	5.92	12.0
10000	5.87	13.5	5.85	14.2
20000	5.82	15.3	5.84	14.3
	OREGON SOIL (NEUTRAL)			
0	6.93	1.15	6.93	1.15
1000	6.80	1.60	6.83	1.50
5000	6.70	2.00	6.70	2.00
10000	6.50	3.15	6.58	2.65
20000	6.40	4.00	6.52	3.00
	ARIZONA SOIL (ALKALINE)			
		Specific alkalinity		Specific alkalinity
0	8.80	63.0	8.80	63.0
1000	8.77	59.1	8.78	60.4
5000	8.52	33.2	8.71	51.3
10000	8.45	28.3	8.65	45.0
20000	8.40	25.0	8.65	45.0

* For figuring specific acidities from pH, see "A simple method for comparing the acidity of different pH values", by R. E. Stephenson. *J. Am. Soc. Agron.*, 1926, 18: 520. See also "Note on specific acidity", by E. T. Wherry, *Ecology*, 1922, 3: 346.

of the acid soil, of course, a replacement reaction, as in the Hopkins method for the determination of the lime requirement, is being dealt with. Here the cation of the salt is replacing the zeolitic hydrogen of the soil with progressive additions of sodium ion. The reason for increased H-ion concentrations when neutral salts are added to acid soils is thus apparent. In the case of neutral soils, especially those high in organic matter or clay, the cation is probably physically absorbed by the colloidal fraction to a greater extent than is the anion. Subsequent hydrolysis under these conditions would yield an increase in H-ion concentration. Upon first thought, the addition of a neutral salt, as sodium chloride, to an alkaline soil carrying considerable amounts of calcium carbonate should result, by double decomposition, in the production of sodium carbonate and calcium chloride in sufficient quantities to cause an increase in *alkalinity* rather than a decrease, but work in this laboratory by the writer and J. F. Breazeale[1] has shown that owing to the greater tendency to the reverse reaction, such a double decomposition takes place but very slightly, yielding a concentration of sodium carbonate seldom greater than 10 to 20 parts per million. Practically all the western calcareous soils carrying "white alkali" contain considerable quantities of replaceable sodium as well as calcium. When such soils are moistened, hydrolysis of these sodium zeolites at once takes place and results in high alkalinities. If now a soluble sodium salt, as sodium chloride, is added, the ionized sodium of the zeolite is immediately forced back (by common ion effect) in proportion to the concentration of sodium ions added. If enough sodium chloride is used, this reverse process will be complete, making the sodium zeolite completely insoluble. The resulting alkalinity will now be due entirely to the hydrolysis of the small quantities of calcium carbonate present in solution. Thus it is never possible, by neutral salt additions, to reduce the alkalinity of a calcareous soil below that of naturally occurring calcium carbonate, which is approximately pH 8.4. This explains the reasons for the decrease in alkalinity that usually results from the addition of soluble sodium salts or from the presence of naturally occurring salts in the calcareous "white alkali" soils of the West.

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[1] Arizona Agr. Exp. Sta. Tech. Bull. No. 6.

In all the writer's experience with "alkali soils" in Arizona, California, and the Hawaiian Islands, he has never seen an "alkali soil" that was acid. In probably 95 per cent of the cases, alkali soils are calcareous, and with this class of soils the explanation of lowered alkalinity by added sodium salts as given above will probably hold. Much additional work along this line was done in the writer's laboratory during the past year, but, as the data in all cases corroborate those tabulated above, they were purposely omitted. Colorimetric determinations with the La Motte H-ion roulette comparator and standards were made in many cases when clarity of the solutions permitted. The two methods usually check closely. The colorimetric method is strongly advised when fairly clear colorless solutions are under investigation, because a great saving of time is assured.

The quinhydrone electrode has recently been advocated as a means of measuring the H-ion concentration of soils by Christensen and Jensen[1] and by Biilmann[2]. Although the writer has had no experience with this electrode, comparisons by others do not always show a satisfactory agreement between it and the hydrogen electrode. A comprehensive article, entitled "The use of the quinhydrone electrode for measuring the hydrogen-ion concentration of soils", by L. D. Baver, has recently appeared[3]. After a number of variable factors have been worked out, the method may give results of sufficient accuracy within a certain pH range. It is not affected by nitrates and other substances which often "poison" the hydrogen electrode. Simplicity of construction and operation and greater speed, as compared with the hydrogen electrode, are claimed for it.

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[1] *Tids. Planteavl*, 1923, **29**: 783. Abs. in *Exp. Sta. Record*, 1924, **51**: 805.

[2] *J. Agr. Sci.*, 1924, **14**: 232.

[3] *Soil Sci.*, 1926, **21**: 167.

RECOMMENDATIONS[1].

The associate referee would recommend the adoption of the following details in connection with the electrometric determination of H-ion concentrations in soils:

1. The proportion of 1 of soil to 5 of CO_2-free distilled water.

2. Continuous or intermittent shaking for a period of approximately 30 minutes *immediately* before taking readings.

3. The use of fresh soils when possible. If not, the condition of the soils should be stated.

4. The use of the colorimetric method when nitrates (NO_3) are present in greater concentrations than 400 parts per million of soil.

5. When pH determinations are made on "white alkali" soils the quantities of sulfates and chlorides present should be given. The pH value of "black alkali" soils usually runs from 9.5 to 11 and is seldom determined other than semi-quantitatively by the use of indicators.

6. Electrolytic hydrogen as put on the market in cylinders under pressure is well suited for use in the electrometric method. It should, however, be washed through solutions of alkaline pyrogallol or alkaline permanganate and finally through distilled water before use.

Future work should be done on the effects of filtering and clarifying solutions for use by the colorimetric method of pH determination. Comparisons between it and the electrometric method should also be recorded. As stated in last year's report, a critical study should be made of methods of reporting results. The pH units used at the present time are exponential values, and direct mental comparisons are almost impossible. The directly comparable "specific acidity" and "specific alkalinity" method, as proposed by Wherry some years ago, appears to be much simpler and more suitable to soil work. The quinhydrone electrode might be studied with profit, for there are certain conditions where neither the hydrogen electrode nor the colorimetric procedure can be used.

REPORT ON LIMING MATERIALS.

By W. M. Shaw (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee.*

The work on liming materials during the past year has been devoted to:

(1) The perfection of a filtration device adapted to all types of limes, and

(2) The influence exerted by impurities in aqueous and aqueous-sucrose solutions. Two devices were worked out in collaboration with the laboratory of the National Lime Association. The associate referee

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**: 62.

and the referee have prepared a manuscript giving a description of the two devices and the results of the studies upon the effect of impurities, which they propose to publish in one of the journals that have wide circulation among industrial chemists, to whom the findings will be of most interest.

It is recommended that recognition be given to the advisability of insuring against the vitiating factor of sulfides in calcined and hydrated limes, pyrite in limestone, and sphalerite in dolamites in the determination of carbon dioxide and that the Committee on Revision of Methods (or the Committee on Revision of Methods of Soil Analysis) be requested to insert such provision in the present method.

REPORT ON FEEDING STUFFS.

By W. F. STERLING (Bureau of Chemistry, Washington, D. C.), *Referee.*

The complete reports of the associate referees will be presented in detail, so it is only necessary to give a general outline of the progress made this year.

The Associate Referee on Stock Feed Adulteration obtained collaborative results on samples containing various quantities of oat hulls. He recommends that the microanalytical method used be adopted as tentative. It is further recommended that the study of microanalytical methods, as related to feeding stuffs, be continued.

The Associate Referee on Mineral Mixed Feeds devoted his time to the preliminary work of selecting suitable methods to submit to collaborative study during the coming year. Methods for the determination of iodine and calcium in mineral feeds are outlined, and it is recommended that these be studied collaboratively.

The Associate Referee on the Determination of Moisture sent out samples for collaborative study by the toluene distillation method and the drying method used for routine work by the collaborator. Results were obtained which seem to justify the associate referee's recommendation that the distillation method be made official.

The Referee on Feeding Stuffs concurs in the recommendation of the associate referees.

REPORT ON STOCK FEED ADULTERATION.

By H. E. GENSLER (Department of Agriculture, Harrisburg, Pa.), *Associate Referee.*

The Referee on Stock Feed Adulteration continued the study of his method for the separation and determination of oat hulls in oat products. Having decided that an excellent test of the value of the method would be the determination of hulls in a mixture of crushed hulled oats and oat hulls rather than in whole oats, he submitted samples of finely ground commercial crushed oats, commercial oat hulls, and a mixture of both. The collaborators were instructed to follow a revised form of last year's method[1]. The following table shows the results obtained by the various collaborators:

ANALYST	GROUND OAT HULLS SAMPLE NO. 1	HULLED GROUND OATS SAMPLE NO. 2	60% GROUND OAT HULLS 40% HULLED GROUND OATS SAMPLE NO. 3
	per cent	*per cent*	*per cent*
1	90.65	4.47	38.35
2	81.85	3.84	35.46
3	84.40	6.20	35.40
4	85.40	6.00	40.10
5	88.97	5.53	38.49
6	89.53	5.98	38.85
7	92.66	8.18	39.66
8	89.02	3.28	34.28
9	89.18	4.59	38.04
10	90.94	6.21	39.15
11	92.70	5.86	39.82
12	93.75	6.43	40.49
13	90.50	5.80	39.65
14	84.30	5.53	38.08
15	85.15	5.80	38.27
16	87.40	5.70	38.00
17	82.20	0.00	30.10
Average	88.21	5.20	37.77

It will be noted that the analysts obtained approximately 10 per cent less than the theoretical quantity of hulls in Sample No. 1, and about 5 per cent more in Sample No. 2. These differences must be due either to impurity of the samples or the failure of the method to produce perfect results.

As it was impossible to obtain a sample of oat hulls that was free from starch, dust, and light-chaff, the discrepancy in Sample No. 1 is undoubtedly due to these impurities. Furthermore, the experience of the referee, as well as the results obtained in last year's work, indicates that it is unlikely that any hull material would be poured out in the supernatant liquid.

Sample No. 2 did not contain more than a trace of hulls. Therefore, it must be concluded that these are plus errors, due to the fact, no doubt, that the residue obtained by the analysts included a trace of hulls, hairs, and brand particles. In last year's work the collaborators obtained an average of 32.02 per cent in a sample of ground whole oats that contained 29-80 per cent of hulls. In this case, the quantity of hulls was determined by separating the hulls from the oats by hand. Since 71 per cent of oats was used, a 5 per cent error would equal 3.56 per cent, which, added to 29.80 per cent, equals 33.36 per cent—a difference of 1.34 per cent over

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[1] *This Journal*, 1927, **10**: 32.

32.36 per cent. Considering the results from either angle, the variance is within reasonable accuracy for methods of this kind. A glance at the figures obtained in the analysis of these samples shows that they check very closely.

The results obtained in the examination of Sample No. 3 are quite close to the quantity of hulls actually added. On the basis of the fact that the oat hulls used were not 100 per cent pure as indicated in the figures for Sample No. 1, less than 40 per cent would be expected, which is the case, the average of all results being 37.77 per cent. It may be noted that in last year's work where the samples contained 29.80 per cent, 33.31 per cent and 40.33 per cent of hulls, the average results obtained by the analysts were 32.02 per cent, 35.16 per cent, and 40.08 per cent, respectively.

It is evident, therefore, that the use of this method enables the microanalyst to make an approximate determination of the quantity of hulls in oat feeds. As stated in previous papers, these methods cannot be expected to have the exactness of chemical methods. They are valuable in obtaining evidence of the amount of adulteration. These figures should be used in connection with results obtained by chemical analyses.

The referee believes that this method can be used in many cases where an approximate determination of the quantity of oat hulls is useful.

RECOMMENDATIONS[1].

It is recommended—

(1) That the method for the determination of oat hulls in oat feeds be made a tentative method.

(2) That the study of microanalytical methods as related to feeding stuffs be continued.

REPORT ON MINERAL MIXED FEEDS.

By H. A. **Halvorson** (State Dairy and Food Department, St. Paul, Minn.), *Associate Referee.*

In the past four or five years, a number of products have been found on the market, which, for want of a better name, may be called mineral feeds. Both the number of brands registered and the variety of mixtures have increased rapidly from year to year. The quantity consumed in some of the states has also increased tremendously. In one state the tonnage sold in 1924 increased 600 per cent over that sold in 1923, and the increase was about 100 per cent in 1925 compared with that of 1924. As a result of the wide-spread sale of these mineral feeds, a demand arose for the feed control officials in the various states to take notice of these products and furnish purchasers with some guide as to their relative value.

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**: 72.

The purpose of these mineral feeds is to supply animals with the mineral constituents that are wholly or partly lacking in the ordinary rations. The three ways by which animals can obtain minerals, aside from the eating of ordinary feeds, are listed as follows:

Class I.—By adding as an ingredient to ordinary feeds 2–3 per cent of a single mineral, such as calcium phosphate or calcium carbonate, or a mixture of these two and others.

Class II.—By feeding a mixture of minerals to which no product having a protein, fat, or carbohydrate value has been added. These mixtures are sometimes simple and contain only three or four ingredients. In other cases they are complex and may contain from 15 to 20 ingredients.

Class III.—Products like those in Class II, but to which has been added a minor percentage, perhaps 10 or 20, of some product of feeding value, such as tankage, oil meal, or any one of a dozen others that might be mentioned. Other products in this class might contain yeast or cod liver oil.

The products mentioned in Class I were on the market long before those mentioned in either Class II or Class III. The products in Class II and Class III evidently developed from the use of minerals in Class I or the ordinary mixed feeds. It was not until minerals as such or slightly diluted with tankage and other products to increase their palatability were sold commercially that it became evident that special regulations for these substances were required.

During the summer of 1924 a special committee of the Association of Feed Control Officials of the United States made a study of the subject of mineral feeds for the purpose of recommending uniform rules for classifying these products. The report of this committee contained the following recommendations for classifying mineral feeds, and these rules have been adopted tentatively by that association.

In order to promote uniformity of registration and labeling of feeds containing minerals, excepting the poultry scratch feeds containing grit and shell, your committee recommends:

(a) That mixed feed containing both feed and mineral ingredients requires in addition to the usual declaration of the chemical feed analysis a declaration of each ingredient contained therein and the minimum percentages of lime (CaO), phosphoric acid (P_2O_5), iodine (I), and salt (NaCl), if same are added.

(b) That mineral feeds containing no organic ingredient do not require the usual chemical feed guarantee, but do require a declaration of each ingredient contained therein and the minimum percentages of lime (CaO), phosphoric acid (P_2O_5), iodine (I), and salt (NaCl), if same are present.

(c) That the mineral ingredients be stated in the common English terms, if any such terms exist.

(d) It being impossible to classify separately the drug ingredients and the mineral ingredients, be it resolved:

(1) That all feeds containing mineral ingredients generally regarded as dietary factors essential for the normal nutrition of animals, and which are sold or represented

for the purpose of supplying these minerals as correctives to rations in which these same mineral factors may be deficient, be classified as mineral feeds.

(2) That all other preparations which are sold or represented for the cure, mitigation, or prevention of disease be classified by this association as drugs or medicines.

The use and enforcement of the above regulations enables both the chemist and the intelligent purchaser to compare the values from the standpoint of the various products offered for sale as mineral feeds. In the states where the regulations have been enforced, analytical problems have been encountered, especially in the determination of lime (CaO) and iodine (I). The addition of numerous ingredients in small quantities to what otherwise may have been a fairly simple mixture causes complications. The basis of most of the mixtures is calcium carbonate, usually in the form of limestone or calcium phosphate, sometimes as bone meal but frequently as rock phosphate or spent bone black, and common salt. Often iodine in the form of sodium or potassium iodide, or what is known as iodized calcium, is added to the extent of less than one-tenth of one per cent. The determination of salt and phosphoric acid in these products has not given the trouble that has been encountered when it is necessary to determine the lime (CaO) or the iodine (I).

DETERMINATION OF IODINE.

Difficulties are usually encountered when attempts are made to extract the very small percentage of iodine from mixtures containing tankage or any other substance high in fat or oil. The following modified method for this determination has been proposed by W. B. Griem of Madison, Wis., for use on mineral feeds. It is suggested that a study be made of this method.

IODINE IN MINERAL FEEDS.

Thoroughly macerate a 10 gram sample in a mortar with about 25 cc. of 80 per cent ethyl alcohol. Filter the mixture on a relatively thick asbestos pad, using only as much suction as is absolutely necessary. Wash the residue with an additional 20–40 cc. of 80 per cent alcohol, and also with the filtrate, thereby insuring a clearer filtrate. Then wash the residue with 25 cc. of water followed by an equal quantity of 80 per cent alcohol. Transfer the filtrate containing the dissolved elemental iodine, CaI_2, NaI, or KI, to a 200 cc. separatory funnel. Acidify with about 2 cc. of concentrated hydrochloric acid and shake thoroughly. After several minutes, add an excess (10–20 cc.) of commercial hydrogen peroxide (3 per cent) and again shake thoroughly. After several minutes, add about 15 cc. of pure carbon disulfide and shake vigorously to extract the iodine. Remove the stopper frequently to relieve the pressure. Drain off the carbon disulfide into an accurately calibrated cylinder and add another portion of carbon disulfide, repeating as before until the carbon disulfide is no longer colored. Mix the several portions of carbon disulfide combined in the graduate to homogeneity and measure accurately. If the carbon disulfide extract is cloudy at this point, centrifuge for several minutes. In a Duboscq colorimeter compare this solution with a standard prepared as follows:

Pipet 10 cc. of potassium iodide solution equivalent to 1.3080 grams of potassium iodide per liter into a separatory funnel, add about 60 cc. of 80 per cent alcohol and 2 cc. of concentrated hydrochloric acid, shake, add 10–20 cc. of hydrogen peroxide, shake, and extract the liberated iodine with carbon disulfide as in the determination. Dilute the combined extracts to 100 cc. in a volumetric flask. Mix thoroughly and use as a standard of comparison. This carbon disulfide standard solution contains 0.1 mg. of iodine per cc. In view of the fact that no data are at hand regarding the permanence of the extract, it is best to use only when freshly prepared.

DETERMINATION OF LIME.

For the determination of lime (CaO) in mineral feeds, the analyst should use a method that will give accurate results when phosphates are present. The method should be rapid and otherwise adapted to the products that are to be analyzed. The removal of the phosphates by precipitation with ammonium molybdate has been tried, but this method is slow and tedious and necessitates using a small sample. A modification of the volumetric method for lime has been proposed by A. O. Olson of St. Paul, Minn., for determining calcium oxide in mineral mixtures. This method, which has proved fairly reliable and convenient, is as follows:

CALCIUM OXIDE IN MINERAL FEEDS.

Grind the sample and take a 2 gram portion, igniting in a muffle to burn off organic matter, if present. Dissolve the residue in dilute hydrochloric acid with the aid of heat. Make up the dissolved material to 250 cc. in a graduated flask, pipet 25 cc. of the clear liquid into a 600 cc. beaker, add ammonium hydroxide in slight excess, and dilute until the volume is about 100 cc. Instead of boiling and filtering off ferric oxide and aluminum oxide at this point, make the solution slightly acid with 0.1 *N* hydrochloric acid, using methyl red indicator. Bring back with 0.1 *N* sodium hydroxide to neutral point as shown by the intermediate or browinsh color of the indicator. Dilute to about 300 cc., boil, and add slowly with constant stirring a solution containing 3 grams of ammonium oxalate, which should also be boiling hot. Let stand overnight to allow the precipitate to settle. Filter and wash thoroughly with cold water. Transfer the paper and the precipitate to a 600 cc. beaker and dilute with water to about 300 cc. Add 5 cc. of concentrated sulfuric acid. Stir well and bring the solution to boiling. While hot, titrate with 0.1 *N* potassium permanganate until a slightly pink color is obtained. Calculate the percentage of calcium in the sample

RECOMMENDATIONS[1].

It is recommended—

(1) That the proposed method for iodine in mineral feeds be studied during the coming year and that samples be submitted to collaborators for analysis.

(2) That the proposed method for lime (CaO) be further studied and that samples be submitted to collaborators for analysis.

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**. 63

REPORT ON DETERMINATION OF MOISTURE.

By F. R. DARKIS (University of Maryland, College Park, Md.), *Associate Referee.*

At the annual convention of the association in 1925, the Bidwell-Sterling[1] distillation method for the determination of water in cattle feeds was adopted as tentative. At this time it was recommended that a further collaborative study be made.

Following this recommendation, uniform representative samples of cottonseed meal, dairy feed, wheat bran, and a mixed feed consisting of one-fourth alfalfa and three-fourths corn meal, by weight, were ground to pass a 20-mesh sieve, thoroughly mixed, and placed in air-tight containers. Subdivisions of these samples were sent to the collaborators.

Accompanying the samples were a reprint of the paper, "Preliminary Notes on the Direct Determination of Moisture"[2], presented at the meeting of this association in 1924, and the suggestions given below. It was requested that determinations for moisture be made by the direct method and also by the method in use in the collaborator's laboratory.

SUGGESTIONS TO COLLABORATORS.

1. Place the toluene and dry sand (if necessary) in the dry flask; then weigh the sample and transfer it into the flask as rapidly as possible to prevent absorbing moisture from the atmosphere. Keep the flask tightly stoppered between the time of weighing the sample and starting the determination, for any additional moisture taken up will affect the final result.

2. Use a condenser with an inner tube, the internal diameter of which is not more than one-half inch.

3. Distil slowly, keeping the condensed column of toluene and water vapors from rising more than 2 inches in the condenser, until most of the water has been distilled over, usually from 30–45 minutes. Then wash down through the top of the condenser with 5–10 cc. of toluene and continue the distillation rapidly for about 5 minutes. Repeat this process until all the moisture is removed.

4. Do not use the brush until well toward the end of the determination, at which time most of the water is in the receiving tube.

Method I in the table refers to the direct method, and Method II to the method in use in the collaborator's laboratory.

Seven of the twelve collaborators submitted the results that are given in the table.

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[1] *J. Ind. Eng. Chem.*, 1925, **17** 147

[2] *This Journal*, 1925, **8** 295.

TABLE 1.

Collaborative results.

COLLABORATORS	DAIRY FEED		MIXED FEED		COTTONSEED MEAL		WHEAT BRAN	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	9.16	9.61	8.74	9.52	7.80	8.56	6.95	8.10
2	10.08	10.16	9.67	9.95	9.25	9.04	8.57	8.53
3	9.47	9.45	9.08	9.10	7.42	8.07	7.87	7.86
4	9.29	9.29	8.75	8.72	8.29	7.65	8.38	7.45
5	10.04	7.91	9.56	7.39	8.74	6.64	8.30	6.57
6	9.98	9.95	9.68	9.73	8.70	8.67		....
7	9.50	9.34	9.25	9.26	8.06	8.00	7.50	7.95
X*	9.70	9.68	9.10	9.27	8.30	8.25	8.25	8.25
Average	9.65	9.42	9.23	9.12	8.32	8.11	7.99	7.82
Difference between the two averages	0.23		0.11		0.21		0.17	
Difference between the highest and lowest result	0.92	2.25	0.94	2.56	1.83	2.40	1.62	1.98

* Associate referee's results

From the results shown, it may be concluded—

1. That for rapid determination of moisture this method has proved very efficient.
2. That from the wide range of results reported by the various collaborators, it is evident that some specific moisture method should be adopted.

It is recommended[1] that this method be adopted as official.

REPORT ON SUGARS AND SUGAR PRODUCTS.

By H. S. PAINE (Bureau of Chemistry, Washington, D. C.), *Referee.*

Considerable delay in getting work under way was caused by resignations of the Associate Referees on Honey, Starch Conversion Products, and Drying, Densimetric, and Refractometric Methods. The Associate Refereeships on Honey and Drying, Densimetric, and Refractometric Methods were filled relatively late in the year by the appointment of H. A. Schuette of Madison, Wisc., and R. T. Balch of Washington, D. C., respectively.

It is believed that the program of work in each of the six sub-divisions has already been sufficiently well outlined to provide for next year's work, and it is recommended that it be continued along the lines already suggested and approved.

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**. 63.

No report on honey was given by the associate referee.

REPORT ON MAPLE PRODUCTS.

By H. M. LANCASTER[1] (Department of Health, Ottawa, Can.), *Associate Referee.*

Maple sirup, as sold or offered for sale in Canada, must be entirely a maple product, but it may be made either by concentrating maple sap to the proper consistency or by dissolving maple sugar in water and evaporating the solution till the correct proportion of solids is obtained. The standards established by the regulations under the Food and Drugs Act make no distinction between these two types of sirup, although the former is more accurately described as maple sap sirup and the latter as maple sugar sirup. Although this reconstructed sirup lacks the bouquet or aroma of maple sirup freshly prepared from sap, it has the greater portion of the genuine maple flavor. Not only is it demanded by trade requirements, largely because of economies in storage and shipment, but it is appreciated by many consumers.

A number of factories purchase the maple sugar by the carload in the eastern section of the Province of Quebec, where it is collected by dealers who obtain it in small lots from the individual producers. As ordinarily marketed, it is in the form of blocks approximately 3 inches by 4 inches by 6 inches that are packed in sacks, each sack weighing about 110 pounds. The quality is exceedingly variable, depending largely upon the care taken in making it in the woods. Some of it is of good color and flavor, but a large proportion of it is dark, almost black, sticky and strong in flavor. It is hardly reasonable for a manufacturer to expect to reconstruct high quality sirup from such sugars. Upon investigating the quality of the product turned out by these factories, it was found that the ash and the Canadian lead number of the sirup as sold were amazingly less than would be expected from the composition of the raw materials used.

Little information has heretofore been available concerning the effect that manufacturing processes have upon the chemical constants of the material. It was considered advisable, therefore, to make a thorough investigation of the whole problem by examining several hundred samples of the commercial sugars as they come to such plants and to ascertain the effects, if any, produced by the factory operations. Table 1 summarizes the results obtained in the analysis of 384 samples of these commercial sugars, collected at the factory from the crops of 1924 and 1925.

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[1] Acknowledgment is hereby given to F. C. Collier, assistant chemist, Department of Health, Ottawa, Can., for assistance in compiling this report.

TABLE 1.

Composition of maple sugar as delivered at factories.

	1924	*1925*
No. of samples	198	186
Canadian lead number—		
Maximum	10.29	12.29
Minimum	2.20	2.19
Average	5.09	5.90
No. of samples less than 3.0	7	6
greater than 8	11	23
Ash (per cent)—		
Maximum	4.37	3.05
Minimum	0.57	0.59
Average	1.12	1.11
Less than 0.75 per cent	6	7

It is evident from the results given in Table 1 that the average of these samples exceeds the generally accepted minima for lead number and total ash. It may be concluded also that although commercial maple sugars are often of inferior grade owing to faulty methods of manufacture in the woods, actual adulteration by the farmer is comparatively rare, being indicated in less than 5 per cent of the samples. The factory managers are inclined to exaggerate the importance of extraneous matter sometimes found in the sugars they buy from the producer. It is true that they have found in them such things as bricks, stones, and horse shoes, but such findings are of rare occurrence. Complaints as to the large quantity of sludge obtained on filtering were likewise found to be largely without foundation because of neglect to allow for clarifiers added and the water present in the moist sludge.

On the other hand, it was not easy to conceive of any explanation that would justify the great difference in the lead number and ash of the sirups as marketed by the factory managers, when compared with the lead number and ash of the sugars used in making the sirup. Many analyses of the sirup manufactured by one company showed a lead number of about 2 and an ash of about 0.6. These findings would certainly not be expected if sugar with a lead number averaging 5 and an ash averaging 1.1 per cent were used. Of course it was suspected that adulteration with cane sugar was being practiced.

In order to reconstruct the sirup from the sugar, it is necessary to redissolve it in water, remove any insoluble solids, and to concentrate by evaporation to remove the excess of water. These are the essential steps, but they are supplemented in most cases by various processes of clarification, sedimentation, and filtration. The factory first investigated makes use of filter presses and vacuum evaporators. In preparing a sirup batch of average size, the weighed contents of ten sacks (approximately 1100 pounds) are dumped into a kettle equipped with both open and closed steam coils and mechanical stirrer. Sufficient water to cover the sugar is added, the steam is turned on, and solution is assisted by mechanical agitation. The liquid is then boiled and skimmed to remove floating matter, and the sugar solution is filtered by pumping through a filter press. In order to assist clarification and to prevent the press from being clogged, finely powdered kieselguhr, equivalent to about 1 per cent of the sugar, is added before filtration. The filtered liquid, containing about 43 to 47 per cent of solids, is concentrated by boiling under reduced pressure at a temperature not exceeding 65°C. in a Pfaudler glass-lined vacuum kettle. Concentration is continued until the sirup reaches over 65 per cent of solids, when it is pumped into an open kettle provided with open and closed steam coils. Here the sirup receives a short boil under atmospheric pressure in order to complete sterilization and to bring it to the desired concentration. It is now ready for transfer to the shipping containers.

Table 2 summarizes the analytical results obtained in the examination of portions of the sirup taken at various steps in the process of manufacture. Batches A and B were made from sugar of the 1924 crop and Batches C and D were made from sugar of the 1925 crop.

TABLE 2.

Results of analyses of sirup made during manufacture.

(Calculated to dry basis.)

SIRUP FROM 1924 SUGAR:	BATCH A			BATCH B		
	Total Solids	Canadian Lead Number	Total Ash	Total Solids	Canadian Lead Number	Total Ash
	per cent		*per cent*	*per cent*		*per cent*
Solution before adding kieselguhr........	41.2	4.17	0.95	46.0	3.98	0.91
Solution after adding kieselguhr............	41.4	3.93	0.89	46.3	3.75	0.91
After filter pressing.....	41.4	4.47	0.85	46.4	4.06	0.80
Leaving vacuum pan....	70.7	4.46	0.89	69.2	3.56	0.74
Finished sirup..	71.5	3.75	0.74	66.5	3.69	0.75
SIRUP FROM 1925 SUGAR:	BATCH C			BATCH D		
Skimming after solution.	....	....	.. .	58.8	....	0.86
Solution before adding kieselguhr	51.8	4.50	1.02	46.6	4.12	0.96
Solution after adding kieselguhr...	51.9	4.56	1.10	46.6	4.42	0.90
After filter pressing.....	52.3	4.02	1.08	47.6	3.92	0.92
Leaving vacuum pan ...	67.3	4.30	0.91	66.1	4.14	0.89
Finished sirup..	67.3	4.36	0.90	65.3	4.26	0.89

CONCLUSIONS.

The results given in Table 2 indicate that the processes of clarification, filtration, and concentration by boiling have only a slight effect upon the chemical constants of the maple sugar sirup. A slight unexplained increase in lead number is noticed in Batches A and B, apparently produced by filter pressing after the press had been set up with fresh cloths.

Insoluble solids constitute from 2.6 to 4.4 per cent only of the sugars as they come from the area of production. Furthermore, the sludge as it came from the press was found to be approximately half water.

Since all results have been calculated to the dry basis, it is logical to conclude that the lead number and ash shown by the sirup should not be much less than the lead number and ash of the sugar from which the sirup is reconstructed.

No report on starch conversion products was made, as no associate referee was appointed.

No report on drying, densimetric, and refractometric methods was made by the associate referee.

REPORT ON POLARISCOPIC METHODS.

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee.*

In last year's report[1] to this association the referee recommended that the investigation on the determination of sucrose be continued and suggested that analysis be made of mixtures containing not only sucrose, invert sugar, and reversion products, but also the ash and organic non-sugars that usually occur in cane products. It was further recommended that the work on the two-enzyme method of Paine and Balch for the determination of sucrose and raffinose in the presence of each other be repeated and extended.

To carry out the first of these recommendations, it was necessary to decide in what form the ash and organic non-sugars were to be added to the sugar mixtures employed in the previous investigations. Theoretically, it would be possible to invert all the sucrose in a low-grade molasses and then add known quantities of sucrose to it; in practice, however, the dark color of the product would preclude accurate saccharimeter readings. Three different methods have been employed to overcome this difficulty. One of these methods made use of artificial mixtures of salts of various inorganic and organic acids and certain nitrogenous materials and gums, but since the actual nature of the non-sugars occurring in cane sirups and molasses is only imperfectly known, such artificial mixtures are not necessarily representative, and any results obtained by their use are of doubtful value.

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[1] *This Journal*, 1926, **9**: 166.

In another method a lead subacetate precipitate, produced in a molasses solution, is filtered off, decomposed by means of hydrogen sulfide or by some acid forming an insoluble lead salt, and the filtrate from the lead sulfide or other lead salt is used to represent the non-sugars. This procedure obtains the non-sugars precipitated by lead subacetate, but it leaves in the original solution others that may be as important as, or even more important than, those precipitated.

The third method requires the complete fermentation of a blackstrap solution, and the utilization of the concentrated residue as the non-sugar mixture. It is quite possible, and even probable, that fermentation not only destroys the sugars but also changes certain organic non-sugars into other compounds that may have entirely different optical properties.

These three methods are thus open to the objection that the non-sugar mixture obtained may not truly represent that actually found in cane sirups and molasses.

In a search for a better procedure, it occurred to the referee to remove the disturbing coloring matter in molasses by the use of an activated carbon. Previous investigations with such carbons had shown[1] that in contact with solutions of cane products they adsorb principally coloring matter and similar colloids present in extremely small quantities, while other non-sugars are removed to a much smaller extent. The result of a carbon treatment of blackstrap is, therefore, a light colored product which contains the same non-sugars as the original material, only in smaller relative quantities.

A supply of blackstrap molasses was kindly furnished by N. Fuad, chemist of the American Molasses Co., and the necessary quantity of activated carbon by Leonard Wickenden, vice-president of the Suchar Process Corporation. The writer wishes to express to both donors his thanks for their courteous cooperation.

PRELIMINARY EXPERIMENTS.

In a preliminary test it was found that 125 per cent of carbon, calculated on solids in molasses, had to be used to bring about the necessary decolorization. This quantity of carbon could not be added at one time, because it would form an unworkable stiff paste with the molasses. It was necessary to dilute the blackstrap to about 20° Brix, and to add the carbon in several successive operations. With the available laboratory equipment it was possible to treat 1 kilogram of molasses at a time. This was dissolved in 3 liters of water, 200 grams of carbon was stirred in, and the mixture was heated to boiling and immediately filtered through filter paper on a Büchner funnel. The cake was washed with hot water until the volume of the filtrate was equal to that of the original solution. More thorough washing would have resulted in too great a quantity of liquid to be evaporated. The treatment with 200 grams of carbon was then repeated four times, 1 kilogram in all being used. The final filtrate was evaporated in vacuo to a density of about 50° Brix, and the resulting sirup was stored in the refrigerator. Altogether 10 kilograms of blackstrap was thus treated. Owing to incomplete washing, less than one-half of the solids in the original material was recovered.

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[1] **Louisiana Bulls. 161 and 173.**

Both the blackstrap itself and the decolorized sirup were then analyzed by the staff of this laboratory. For the sucrose determination, the blackstrap was clarified with dry subacetate of lead, the solution was deleaded with ammonium dihydrogen phosphate, and the hydrolysis was carried out by means of invertase. In the analysis of the decolorized sirup the invertase method was used without any previous clarification, as the normal solution could easily be read in a 200 mm. tube. After clarifying with neutral lead acetate and deleading with potassium oxalate, reducing sugars were determined in both products by means of Allihn's method, Browne's conversion factor being used for invert sugar and his correction formula for the reducing effect of sucrose. The ash is sulfated ash less one-tenth. The results of the analysis, expressed in percentage, are given in Table 1.

TABLE 1.

DETERMINATION	ORIGINAL BLACKSTRAP	SAME, ON BASIS OF 100% SOLIDS	DECOLORIZED BLACKSTRAP	SAME, ON BASIS OF 100% SOLIDS
Total solids by refractometer	81.70	100.00	48.90	100.00
Sucrose	32.53	39.82	17.17	35.11
Invert sugar	20.86	25.53	19.38	39.63
Ash	9.33	11.42	6.72	13.74
Organic non-sugars	18.98	23.23	5.63	11.51
Purity, expressing total sugars as sucrose	...	64.08	...	72.76

Comparison of the analyses shows that considerable inversion took place during the process owing to the repeated boilings at low density. However, owing to the effect of the carbon in removing non-sugars, the total sugars, expressed as sucrose, increased from 64.08 per cent of the total solids to 72.76 per cent. The ash increased because the carbon had very little adsorptive effect on the principal ash constituents. The organic non-sugars in the decolorized material are about one-half the quantity present in the original blackstrap.

The preparation of this decolorized sirup required so much of the referee's spare time that he was unable to advance the work to a stage where the analytical work recommended in last year's report could be started. It is intended now to invert all the sucrose in the sirup by means of invertase, and then to apply the various inversion methods to mixtures of sucrose with this non-sucrose sirup.

RECOMMENDATION[1].

It is recommended that the work outlined in Recommendations (4) and (5) in the associate referee's report for 1925 and not finished during the present year, be completed during the coming year.

No report was made on chemical methods for reducing sugars by the associate referee.

COMMITTEES NAMED BY THE PRESIDENT.

Committee to Wait upon Secretary of Agriculture: W. W. Skinner, W. H. MacIntire, and G. S. Fraps.

Committee to Wait upon Honorary President: B. B. Ross and R. N. Brackett.

Committee on Resolutions: A. S. Mitchell, J. W. Kellogg, and H. C. Fuller.

Auditing Committee: H. B. McDonnell and J. B. Weems.

Committee on Nominations: Julius Hortvet, A. J. Patten, and F. C. Blanck.

FIRST DAY.

MONDAY—AFTERNOON SESSION.

REPORT ON FERTILIZERS.

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.), *Referee.*

The recommendations of the associate referees this year are chiefly for further work.

The question of cancelling the calcium chloride method for preparing ammonium citrate was discussed last year, and no objection to this action was taken. The referee, therefore, makes this recommendation.

The neutral and permanganate methods for nitrogen in fertilizers have been discussed recently, and it appears that some changes should be made in them. It is possible that the method of washing should be changed, that the approximate time of distillation should be stated in the alkaline permanganate method, and that the use of paraffin in the distillation be omitted. It is also possible that other details should be more definitely described. It would probably be best for the Associate Referee on Nitrogen to consider this matter and make the necessary recommendations. A review of the A. O. A. C. work on this subject by E. C. Carlyle and the referee follows this report.

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**: 64; 1926, **9**: 73.

Some requests have been made for changes in methods that would give higher results for certain products. One request was that in case of high-analysis phosphates 1 gram be used instead of 2 grams as a sample for the determination of available phosphoric acid, because these phosphates contain 6 to 8 per cent citrate-soluble phosphate, which saturates the ammonium citrate. No evidence that such saturation occurs was presented. When it was pointed out by the referee that some acid phosphates contain more than this quantity of citrate-soluble phosphoric acid, nothing further was said on this point. This change was again requested for the reason that when the fertilizer in question, which contained about 1.60 per cent insoluble phosphoric acid, was mixed with other materials, about 0.10 per cent more available phosphoric acid was obtained in the mixtures. The actual results presented were as follows:

	per cent
Citrate-insoluble phosphoric acid, using 1 gram	1 46
Citrate-insoluble, using 2 grams	1 08
Citrate-insoluble in mixture, 500 pounds phosphate to the ton	0 32

These differences appear to the referee to be entirely too small to justify asking for any change in the method.

Letters were also received stating that since calcium potassium phosphate was prepared by fusion, the Wagner (citric-acid soluble) method for Thomas phosphate should be applied to it, especially as this method gives higher results. The citric-acid method is an empirical method that has been found satisfactory when applied to Thomas phosphate, but it does not necessarily apply to other products made by fusion. The use of the method could be justified only in case results obtained in pot and field experiments similar to those made on Thomas phosphate should show it to be applicable to the product concerned. Furthermore, calcium-potassium phosphate is not a by-product; it is made by heating potash salts with phosphate rock and probably contains unaltered calcium phosphate.

The referee believes that requests for changes similar to those mentioned should be accompanied by evidence that the higher analytical result is justified by a higher agricultural value of the portion of the fertilizer concerned.

The referee is not authorized to make any rulings concerning such matters; he can only bring them to the attention of this association with such recommendations as appear justified by the evidence presented.

RECOMMENDATIONS[1].

It is recommended—

(1) That the absolute or cupric oxide method for nitrogen[2] be removed from the methods for fertilizers.

(2) That the calcium chloride method for preparing ammonium citrate[3] be eliminated from the methods.

(3) That the neutral and alkaline permanganate methods for nitrogen[4] be considered by the Associate Referee on Nitrogen, and that such modifications as appear desirable be recommended.

(4) That the recommendations of the associate referees be endorsed.

REVIEW OF A. O. A. C. WORK ON ALKALINE AND NEUTRAL PERMANGANATE METHODS FOR NITROGEN.

(Compiled by S. E. CARLYLE and G. S. FRAPS.)

1896 (1)[5]. J. P. Street, referee, recommended the Hayes method of alkaline permanganate for study and trial. This method (2) uses 1 gram of sample and 100 cc. of permanganate. The results were not concordant. No paraffin was used.

1897 (3). J. P. Street, referee, used a small piece of paraffin. Slade introduced a modification requiring 100 cc. of 3 per cent permanganate on a 1 gram sample.

1898 (4) (5). C. H. Jones presented his alkaline permanganate method, which requires digesting for 1 hour below boiling and then distilling until digestion is complete and the use of 45 mg. of nitrogen. No paraffin was used.

1899 (6). F. S. Shiver, referee, used in the neutral permanganate method 75 mg. of nitrogen and 100 cc. of 1.6 per cent permanganate. In the alkaline permanganate method there was no mention of paraffin. The material was digested 1 hour and distilled for 1 hour.

1900 (7). W. R. Perkins, associate referee, studied the neutral permanganate method with several modifications, as washing down the flask after 10 minutes' digestion, adding water, filtering, and washing to 200 cc. No study was made of the alkaline permanganate method.

1901 (8). W. R. Perkins, referee, recommended the neutral permanganate as a provisional method. Used 75 mg. of nitrogen and 125 cc. of 1.6 per cent permanganate. The alkaline permanganate was recommended for further study.

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[1] For report of Sub-committee A and action he association, see *This Journal*, 1927, **10**: 64.

[2] *Methods of Analysis, A. O. A. C.*, 1925, 9.

[3] *Ibid.*, 4, Sec. 13(2).

[4] *Ibid.*, 12.

[5] Numbers refer to the list of references.

1901 (21). Jones and White published an article in the 14th Annual Report of the Vermont Agricultural Experiment Station, 1901, describing laboratory method for organic nitrogen availability.

1901 (22). J. P. Street published his article on availability of organic nitrogen.

1902 (9). F. W. Morse, referee. The neutral permanganate method remained as in 1901. The alkaline permanganate method required 45 mg. of nitrogen, digestion for 1 hour, and distillation till complete. No paraffin was used.

1903 (10). F. W. Morse, referee, used the two methods employed in 1902, but he makes the first mention of a definite quantity of distillate—100 cc. in the alkaline permanganate method to be tried as a modification. No paraffin was used.

1904 (11). C. H. Jones, referee. The alkaline permanganate method distilled 85 cc. after 30 minutes' digestion. No paraffin was used. Nothing was done on the neutral permanganate method.

1905. No work was done on the method.

1906 (12). J. H. Gibboney, referee, modified the alkaline method, using 0.0675 gram of nitrogen and 150 cc. of alkaline permanganate and distilling to 100 cc.

1907–8–9. No work was done.

1910 (13). C. H. Jones, referee, changed the alkaline permanganate method, using 50 mg. of nitrogen and distilling to 95 cc. The neutral permanganate method was changed to 45 mg. of nitrogen and 2 per cent permanganate.

1911. No work was done.

1912 (19). C. H. Jones, referee, gave an article describing the alkaline permanganate method in detail. See also C. H. Jones, Vermont Experiment Station Bulletin 173 (20).

1912. No work was found.

1913 (14). C. L. Hare, referee, tried both methods, using 50 mg. of nitrogen and in the alkaline method glass beads to prevent bumping, but no paraffin.

1914 (15). R. N. Brackett and H. D. Haskins, referees, in the neutral permanganate method used 45 mg. of nitrogen instead of 50, as in 1913. The alkaline method does not use paraffin. Both methods were recommended and adopted as official (16). B. B. Ross was chairman of Committee A.

1915 (17). R. N. Brackett and H. D. Haskins, referees, used a piece of paraffin in the alkaline method and distilled 95 cc.; 50 mg. of nitrogen was used in neutral permanganate instead of 45, as in 1914.

1916 (18). W. W. Skinner, chairman Committee A, recommended that no further work be done on these methods. Since then no additional official work has been done.

E. W. Magruder (23) made a study of the alkaline permanganate method with the collaboration of several chemists.

C. S. Robinson (24) wrote an article, entitled "Interpretation of Results of Analysis by Permanganate Method".

Gordon Hart (25) made a study of the size of the sample to be used in the neutral permanganate method.

REFERENCES.

(1) U. S. Dept. Agr. Div. Chem. Bull. 49, p. 25. 1896.
(2) Ibid., p. 24, 1896.
(3) Ibid., 51, p. 23. 1897.
(4) Ibid., 56, p. 21. 1898.
(5) *This Journal*, 1920, **3**: 289.
(6) U. S. Dept. Agr. Div. Chem. Bull. 57, p. 51. 1899.
(7) Ibid., 62, p. 17. 1900.
(8) Ibid., 67, p. 83. 1901.
(9) Ibid., 73, p. 49. 1902.
(10) Ibid., 81, p. 83. 1903.
(11) Ibid., 90, p. 120. 1904.
(12) Ibid., 105, p. 76. 1906.
(13) Ibid., 137, p. 14. 1910.
(14) *This Journal*, 1915, **1**: 17.
(15) *Ibid.*, 380.
(16) *Ibid.*, 1916, **2**: 42.
(17) *Ibid.*, 1917, **3**: 207.
(18) *Ibid.*, 520.
(19) *J. Ind. Eng. Chem.*, 1912, **4**: 438.
(20) Vermont Agr. Exp. Sta. Bull. 173.
(21) Report of the Vermont Agr. Exp. Sta., 1901, p. 219.
(22) *J. Am. Chem. Soc.*, 1901, **23**: 330.
(23) *This Journal*, 1920, **5**: 454.
(24) *Ibid.*, 1924, **7**: 373.
(25) *Ibid.*, 379.

THE GRAVIMETRIC DETERMINATION OF PHOSPHORIC ACID.

By WILLIAM H. ROSS (Bureau of Soils, Washington, D. C.), *Associate Referee.*

At last year's meeting of this association a report[1] was given on a collaborative study of the official method for the gravimetric determination of phosphoric acid. In this study special attention was given to the effect on the results of varying the conditions under which precipitation is made with magnesia mixture. Standard phosphate samples were submitted to fourteen collaborators, and reports were received from eleven. One of the samples was the standard phosphate rock of the

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[1] *This Journal*, 1926, **9**: 182.

Bureau of Standards, and the other was a synthetic calcium phosphate prepared by the associate referee.

The directions that accompanied the samples called for precipitation of the ammonium magnesium phosphate under four different conditions, viz.: by adding the official alkaline magnesia mixture to (1) alkaline, (2) neutral, and (3) acid solutions; and (4) by adding acid magnesia mixture to an acid solution and then making alkaline with ammonia, as recommended by Lundell and Hoffman[1] of the Bureau of Standards.

The results reported by some of the collaborators varied considerably from the theoretical, but eight of the eleven reported at least one value for each sample that agreed within 0.2 per cent of the assumed true values of the samples. A comparison of the mean of the results reported by these eight collaborators for the different procedures with the true values of each sample showed that in the hands of these collaborators lowest results are obtained with alkaline solutions and highest with acid, but the differences are not great, and both neutral and acid solutions give results that agree very well with the theoretical. It was concluded, therefore, that while variations in the reaction of the solution before precipitating with magnesia mixture[2] may be a contributing source of error, it is not the principal cause of the wide variations in the results found by different analysts.

In continuing the work for the present year, standard phosphate samples were again prepared and sent to fourteen collaborators. Sample No. 1 contained a known quantity of monoammonium phosphate mixed with known quantities of phosphorus-free calcium carbonate, iron oxide, and kaolinite. These products were added with a view to supplying some of the constituents occurring in phosphate rock. Sample No. 2 also contained calcium carbonate in mixture with potassium phosphate. Both phosphates were prepared by recrystallizing the C. P. salts three times, and all materials were dried and ground to 100 mesh before using. The moisture content of the samples after mixing amounted to 0.12 and 0.04 per cent, respectively.

The directions sent to the collaborators differed from those of last year in that Jörgensen's method[3] (Procedure No. 4), as now used in Germany, was substituted for the procedure of making alkaline before precipitating with magnesia mixture, as it is now recognized that this procedure gives low results.

DIRECTIONS FOR COLLABORATIVE WORK.

Determine total phosphoric acid in each of the two samples submitted by the following procedures:

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[1] *This Journal*, 1924, 8: 184.

[2] In the last edition of *Methods of Analysis* an error occurs in the directions given for the preparation of magnesia mixture (p. 2, 5(C)) The first of the alternative methods outlined gives an alkaline and the second a neutral magnesia mixture. The use of the latter would not give accurate results in the determination of phosphoric acid by the official method, and the wording *dilute to* 1 *liter* should be changed to read *proceed as in* (1).

[3] *Analyst*, 1926, 51: 61.

(1) Dry sample at 105°C. for 1 hour. Dissolve 2 grams of the sample in 30 cc. of hydrochloric acid (sp. gr. 1.19) and 10 cc. of nitric acid (sp. gr. 1.42), and evaporate to dryness or to a sirupy consistency. Redissolve in 5 cc. of concentrated nitric acid and 50 cc. of water. Cool the solution, make up to the mark in a graduated flask, allow to settle, or pour on a dry filter, and proceed as described in the official gravimetric method for the determination of total phosphoric acid until the molybdate precipitate is washed with ammonium nitrate solution. Then dissolve the precipitate on the filter with dilute ammonium hydroxide (100 cc. ammonium hydroxide (sp. gr. 0.90) per liter of water) and wash with hot water into a beaker to a bulk of not more than 100 cc. Carefully neutralize the ammoniacal solution with hydrochloric acid, using litmus paper or brom thymol blue as indicator, cool, and from a buret add drop by drop with stirring, 15 cc. of the official alkaline magnesia mixture for each decigram of phosphoric acid (P_2O_5) present. After 15 minutes, add 12 cc. of strong ammonia. Let stand for at least 4 hours, filter through paper, and wash with the dilute ammonia until the filter and precipitate are free from chlorides. Transfer filter and precipitate to a platinum or porcelain crucible, dry without charring, burn below redness, and ignite—preferably n an electric furnace—to constant weight at 1000°–1050°C. Cool in a desiccator and weigh as magnesium pyrophosphate ($Mg_2P_2O_7$). (Official method.)

(2) Proceed as in (1) through the point where the solution of the molybdate precipitate is carefully neutralized, and then make acid by the addition of 1 cc., or its equivalent, of hydrochloric acid (sp. gr. 1.19). Proceed with the addition of the official magnesia mixture and complete the determination as in (1). (Proposed modification of official method.)

(3) Prepare acid magnesia mixture as follows: Dissolve 50 grams of magnesium chloride and 100 grams of ammonium chloride in 500 cc. of water. Add ammonium hydroxide in slight excess, let stand overnight, and filter if a precipitate appears. Make barely acid with hydrochloric acid and dilute to 1000 cc.

Prepare a solution of the sample and proceed as in (1) through the point where the solution of the molybdate precipitate is carefully neutralized. Add 1 cc., or its equivalent, of hydrochloric acid (sp. gr. 1.19) and 10 cc. of acid magnesia mixture per decigram of phosphoric acid (P_2O_5) present. Add ammonium hydroxide (sp. gr. 0.90) dropwise and with continuous stirring until the solution is ammoniacal, and most of the phosphoric acid has been precipitated. Finally add 15 cc. more of ammonium hydroxide at one time, let stand for 4 hours or overnight at room temperature, and complete the determination as in (1). (Lundell and Hoffman's modified routine method.)

(4) Prepare neutral magnesia mixture as follows: Dissolve 50 grams of magnesium chloride and 150 grams of ammonium chloride in water and make up to 1000 cc.

Prepare a solution of the sample and proceed as in (1) through the point where the molybdate precipitate is dissolved in dilute ammonia and washed with hot water into a beaker. Heat the alkaline solution thus obtained to the boiling point, and without cooling, add slowly from a buret drop by drop with constant stirring 20 cc. of neutral magnesia mixture per decigram of phosphoric acid (P_2O_5) present. Allow to stand without any extra addition of ammonia for at least 4 hours, filter, and complete the determination as in (1). (Jörgensen's method.)

SUGGESTIONS.

The presence of unburnt carbon in the ignited residue is also claimed to be a source of error in the analysis of phosphates. It is possible to obtain a residue that will burn snow white throughout, but under certain conditions a dark colored residue that retains unburnt carbon after prolonged ignition may be obtained. Careful note, therefore, should be made if any of the procedures give a residue that fails to burn white throughout the whole mass.

The claim has been made that the occurrence of molybdenum in the magnesium pyrophosphate residue is a source of error in the gravimetric determination of phosphates. The quantity actually present can be easily determined by the following method of McCandless and Burton[1] and it is accordingly suggested that this determination be made if it is convenient to do so.

COLORIMETRIC METHOD FOR THE DETERMINATION OF MOLYBDIC ACID (MoO_3) IN MAGNESIUM PYROPHOSPHATE RESIDUES.

REAGENTS.

(a) *Standard molybdate solutions*.—Dissolve 0.5 gram of molybdenum trioxide (99.9 per cent reagent) in hot, dilute ammonia solution, boil to expel excess of ammonia, and make up to 250 cc. Take an aliquot of this solution and dilute to 20 times its volume to form a solution that contains 0.1 mg. of molybdic acid (MoO_3) per cc.

(b) *Sodium sulfide solution*.—Dissolve 10 grams of sodium sulfide crystals in 100 cc. of water.

DETERMINATION.

Prepare a solution of the magnesium pyrophosphate residue obtained in the gravimetric determination of phosphoric acid by adding 10 cc. of dilute hydrochloric acid (1 : 1) and digesting on a hot plate until the residue dissolves. Filter, if necessary, and wash into a 100 cc. flask, make up to the mark, take an aliquot of 10 cc., place in a small porcelain casserole, or evaporating dish, and add 5 drops of the sodium sulfide solution. Molybdic acid, if present, will darken the solution in proportion to the quantity present and can be readily determined by comparing the depth of color with that given by a known quantity of the standard in a solution of the same volume and containing the same quantity of hydrochloric acid.

ANALYSIS OF STANDARD PHOSPHATE SAMPLES.

The results obtained by the collaborators who completed the analyses of the samples a short time after they were prepared are given in Table 1.

As shown in Table 1, the best results were again obtained when an alkaline magnesia mixture was added to a neutral solution, as in the official method, and the corresponding procedures fall in exactly the same order this year as last when arranged according to accuracy.

Several months after the standard samples had been prepared it was observed that a reaction between the phosphate and calcium carbonate in each sample had taken place on standing. All collaborative work on the samples was accordingly stopped, and new standard samples were prepared, but these were not sent to the collaborators owing to lack of time. Sample No. 1 in the new set of standards was prepared by mixing equal parts by weight of monoammonium phosphate and potassium sulfate; Sample No. 2 contained pure monopotassium phosphate only; and Sample No. 3 consisted of a mixture of 15 parts of monopotassium phosphate with 4 of feldspar and 1 of calcium fluoride. The diluting materials were added with a view to supplying some of the constituents occurring in natural phosphates, and so far as known they do not react with the alkali phosphates in a dry state. The results

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[1] *Ind. Eng. Chem.*, 1927, **19**: 406.

TABLE 1.

Analysis of standard phosphate samples.

COLLABORATORS	PHOSPHORIC ACID (P_2O_5) FOUND								
	Sample No. 1*				Sample No. 2†				
	Official Method. Reaction of solution before precipitating with magnesia mixture		Lundell and Hoffman's modified routine method	Jörgensen's Method	Official Method. Reaction of solution before precipitating with magnesia mixture		Lundell and Hoffman's modified routine method	Jörgensen's Method	Mean MoO_3 in ignited residue expressed as per cent of sample
	Neutral	Acid			Neutral	Acid			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A. R. Merz Bureau of Soils	32.19	32.43	32.40	32.21	41.35	41.44	41.49	41.43	Trace
F. A. Barker Federal Phosphorus Co.	32.19	32.64	32.60	32.69	41.01	41.45	41.54	41.51	0.04
D. S. Reynolds Bureau of Soils	32.12	32.20	32.28	32.14	41.47	41.39	41.57	41.47	0.01
A. H. Allen Virginia-Carolina Chemical Corporation	32.44	32.51	32.47	32.44	41.63	41.61	41.65	41.61	Trace
Mean	32.24	32.44	32.44	32.37	41.36	41.47	41.56	41.50	

* Phosphoric acid (P_2O_5) present—32.14 per cent.
† Phosphoric acid (P_2O_5) present—41.29 per cent.

obtained by the associate referee in the analysis of these samples is given in Table 2. The samples were analyzed by Lundell and Hoffman's double precipitation method in addition to the procedures listed in Table 1.

TABLE 2.

Analysis of standard phosphate samples.

(By associate referee.)

SAMPLE NO.	PHOSPHORIC ACID (P_2O_5) PRESENT	PHOSPHORIC ACID (P_2O_5) FOUND				
		Official Method. Reaction of solution before precipitating with magnesia mixture		Lundell and Hoffman's Method		Jörgensen's Method
		Neutral	Acid	Single precipitation	Double precipitation	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	30.86	30.85	30.97	30.92	30.87	30.97
2	52.18	52.26	52.66	52.28	52.18	52.28
3	39.14	38.98	39.26	39.31	39.19	39.39

The results given in Table 2 again support the conclusions of last year and also the claims of Lundell and Hoffman regarding the accuracy of their double precipitation method. None of the residues obtained in these analyses contained more than a trace of molybdenum and all burned perfectly white.

STANDARDS FOR PHOSPHATE ANALYSIS.

A review of the literature shows that many of the phosphate standards used in the past must have been unstable, and it is probable that much of the controversy that has taken place over methods of phosphoric acid analysis has been due to the use of standards of uncertain composition. Replies received in response to a questionnaire sent to all the collaborators showed that one recommended microcosmic salt as a standard, two suggested the use of a solution of crystallized phosphoric acid, while the remaining twelve favored monopotassium phosphate. This salt has been used as a standard both in this country and abroad and is recommended by Jörgensen as the best control preparation. It crystallizes readily and is now prepared in a high state of purity for use as a buffer in hydrogen-ion work; it has no water of hydration; it is one of the least hydroscopic of the soluble salts; and its composition can be checked by igniting to the metaphosphate. The work of the year, however, shows that there are certain salts with which it reacts in the dry state, but there should be no difficulty in selecting diluting materials with which it will not react.

IGNITION OF AMMONIUM MAGNESIUM PHOSPHATE PRECIPITATES.

Several of the collaborators in last year's work reported that some of their precipitates failed to burn white, and that when such was the case the results were usually high. A study of the matter was accordingly undertaken in cooperation with the McCandless Laboratory. It was found that portions of ammonium magnesium phosphate precipitates prepared by the associate referee, and which burned snow white throughout when ignited for one hour at 1000°C. in an electric muffle furnace, failed to burn white when sent to the McCandless Laboratory and ignited at a presumably equal temperature over a gas burner. Likewise, portions of precipitates prepared in the McCandless Laboratory, and which also failed to burn white when ignited in the way described, gave a perfectly white residue when ignited in an electric furnace. It is concluded, therefore, that the failure to obtain a white residue in these tests was due to the manner of igniting the precipitate rather than to any preceding step in the determination, and that improper igniting of the precipitate is an important source of error in the analysis of phosphates.

The work of the year may be summarized as follows—

(1) The quantity of molybdenum in magnesium pyrophosphate residues that have been properly prepared and ignited in an electric muffle furnace for 1 hour at 1000°C. is not sufficiently great to have any appreciable effect on the results.

(2) Ammonium magnesium phosphate precipitates that have been properly prepared and washed will burn to snow whiteness throughout when ignited for 1 hour at 1000°C. in an electric furnace. When precipitates of this kind fail to burn white the result is due to improper igniting rather than to any preceding step in the determination, and the results are usually too high.

(3) So far as they go, the collaborative results support the conclusions of last year and also the claims of Lundell and Hoffman regarding the accuracy of their double precipitation method, but inasmuch as the composition of the standard used in the work changed sonewhat on standing, it is recommended:

RECOMMENDATIONS[1].

(1) That the study of the effect of varying the conditions under which precipitation is made with magnesia mixture be continued for another year.

(2) That the recommendations of the collaborators be followed in the preparation of the standards used in this work.

(3) That the standard, or standards, so prepared be tested at intervals over a sufficient period to insure the constancy of their composition.

(4) That the words "dilute to 1 liter" in the second of the alternative methods[2] for the preparation of magnesia mixture be changed to read "proceed as in (1)".

REPORT ON NITROGEN[3].

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee.*

The work this year consisted in studying methods for the determination of nitrate nitrogen in mixed fertilizers. The investigational work carried on last year by the associate referee[4] indicated that when organic materials were present in a mixed fertilizer, nitrate nitrogen determinations by the official methods[5] were apt to give high results. As a remedy for this condition, a slight modification of the official method suggested some time ago by J. E. Breckenridge[6] was tried out last year with fair success. Further studies on this modified method were carried on this year. However, it was clearly shown in last year's work and by the studies of past investigators that when compounds such as calcium cyanamide and urea are present in mixed fertilizers, all the available methods for determining nitric nitrogen are inaccurate. Since these compounds are being used quite extensively in commercial fertilizers at the present time, it is important that a new and accurate method be devised to measure nitrate nitrogen in the presence of such materials. C. H. Jones of the Vermont Agricultural Experiment Station worked out a method[1] to meet these conditions and, in collaboration with the associate referee, conducted a preliminary investigation. The results were so promising that it was decided to carry on an extensive collaborative study of the method at once.

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[1] For report of Sub-committee A and action of the association, see *This Journal*, 1926, 10: 64.

[2] *Methods of Analysis, A. O. A. C*, 1925, 2, 5(C).

[3] Paper No. 213 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

[4] *This Journal*, 1926, 9: 187.

[5] *Methods of Analysis, A. O. A. C.*, 1925, 11.

[6] *Ind. Eng. Chem.*, 1925, 17 :95.

Four samples of mixed fertilizers were prepared. Sample No. 1 contained nitrate of soda, acid phosphate, and muriate of potash, and Sample No. 2 contained the same materials with the addition of tankage. The Breckenridge modification method was tried out on these two samples in comparison with the official method. Sample No. 3 contained nitrate of soda, calcium cyanamide, acid phosphate, and muriate of potash, while Sample No. 4 had urea in place of the cyanamide. The Jones method was tried out on Samples No. 3 and 4 in comparison with the official method. These samples were carefully prepared and sent out to twenty-five chemists that had signified their willingness to cooperate. Twenty reports were received.

INSTRUCTIONS TO COLLABORATORS.

NITRIC AND AMMONIACAL NITROGEN REDUCED IRON METHOD—OFFICIAL.

DETERMINATION.

Place 1 gram of the sample in a 500 cc. flask, add about 30 cc. of water and 2–3 grams of reduced iron, and, after allowing the mixture to stand sufficiently long to insure solution of the soluble nitrates and ammonium salts, add 10 cc. of dilute sulfuric acid (1 + 1). Shake thoroughly, place a long-stemmed funnel in the neck of the flask to prevent mechanical loss, and allow to stand until the violence of the reaction has moderated. Heat the solution slowly, boil for 5 minutes, and cool. Add about 100 cc. of water, a little paraffin, and 7–10 grams of magnesium oxide, free or nearly free from carbonates. Connect the flask by means of a Kjeldahl connecting bulb with a condenser, such as is used in the Kjeldahl method, and boil the mixture nearly to dryness (about 40 minutes); collect the ammonia in a measured quantity of standard acid; and titrate with standard alkali solution, using cochineal or methyl red indicator. The nitrogen obtained represents the nitrates plus the ammonium salts contained in the sample.

BRECKENRIDGE MODIFICATION.

DETERMINATION.

Place 8 grams of the sample in a 200 cc. volumetric flask, fill to the mark with distilled water, shake thoroughly, and filter. Take 25 cc. aliquots (equal to 1 gram) for analysis and proceed as in the regular reduced iron method.

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[1] *Ind. Eng. Chem.*, 1927, 19: 269.

C. H. JONES METHOD FOR DETERMINING NITRATE NITROGEN IN FERTILIZERS CONTAINING CYANAMIDE, UREA, ETC.

PREPARATION OF SOLUTION.

Place 4 grams of the sample in a 150 cc. beaker, add 40 cc. of water, stir, filter by decantation, and after all the residue is transferred to the filter, wash to just under the 200 cc. mark, letting each washing run through before another is added. Make up exactly to 200 cc., mix, and treat 25 cc. aliquots (equal to 0.50 gram) as follows:

DETERMINATION.

A. Nitric Nitrogen.

(Aliquots for A and B must be taken from the same solution and run at the same time.)

Place 25 cc. of the prepared sample in a 500–600 cc. Kjeldahl distillation flask, add 25 cc. of water, 10–12 perforated glass beads (3–5 mm.), 2 grams of reduced iron, and 10 cc. of dilute sulfuric acid (1 + 1). Rotate slowly and when violence of reaction (if any) has moderated, place on the hot plate and boil gently for 5 minutes. Remove, add 40 cc. of water, and cool. Now add 100 cc. of strong sodium hydroxide solution, 42° Baumé (525 grams of sodium hydroxide in 1 liter of water). Connect at once with a condenser by means of a Kjeldahl connecting bulb, and boil until 150–160 cc. has distilled over and the distillate as it drops from the condenser shows neutral to litmus paper. Collect the ammonia in a measured quantity of standard acid and titrate with standard alkali, using cochineal or methyl red indicator.

The nitrogen obtained represents that from nitrates and ammonia salts, and from other nitrogenous compounds, proteins, cyanamide, urea, etc., that have been changed, wholly or partially, to ammonia by this treatment.

B. Corrective Blank.

With another 25 cc. of the prepared sample, repeat the determination, omitting the addition of reduced iron.

The nitrogen obtained represents that from ammonia salts and other nitrogenous compounds, proteins, cyanamide, urea, etc., that have been changed, wholly or partially, to ammonia by this treatment.

The percentage of nitrogen obtained by procedure A minus that obtained by procedure B equals the percentage of nitrogen from nitrates contained in the sample.

NOTES.

(1) When ammoniacal nitrogen alone is to be determined in the sample, an aliquot prepared as directed is taken, 150 cc. of water and 5 grams of magnesium oxide (heavy) are added, and distillation is continued in the usual manner.

(2) To prevent caking, add water to the flasks at the completion of the process, before cooling and washing.

(3) The addition of perforated glass beads insures gentle boiling, free from bumping.

(4) From 50 minutes to 1 hour is usually required for the distillation of A and B.

(5) During distillation the flasks should rest on asbestos-coated wire gauze.

EXPERIMENTS.

Series I. Run each of the four samples by the reduced iron method (official) as described in the accompanying directions. Run in triplicate.

Series II. Run the Breckenridge modification on Samples No. 1 and No. 2 only, as described in the accompanying directions. Run in triplicate.

Series III. Run the Jones method on Samples No. 3 and No. 4 only, as described in the accompanying directions. Each determination is made in two parts (A and B). Run in duplicate or, if time permits, in triplicate.

The collaborators were the following:

1. L. S. Walker, Massachusetts Agricultural Experiment Station, Amherst, Mass
2. A. O. Olson, Dairy and Food Department, St. Paul, Minn.

3 and 4. W. D. Richardson, Swift and Co., Chicago, Ill.

5. R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
6. M. P. Etheredge and A. D. Andrews, Mississippi State Chemical Laboratory, A. & M. College, Miss.
7. H. L. Moxon, Virginia-Carolina Chemical Co., Richmond, Va.
8. A. D. Rich, Armour and Co., Chicago, Ill.
9. L. A. Salinger, U. S. Department of Agriculture, Bureau of Chemistry, Savannah, Ga.
10. O. B. Winter, Agricultural Experiment Station, East Lansing, Mich.
11. M. G. Musselman, Agricultural Experiment Station, East Lansing, Mich.
12. J. E. Breckenridge, American Agricultural Chemical Co., Carteret, N. J.
13. H. R. Allen, Kentucky Agricultural Experiment Station, Lexington, Ky.
14. L. J. Jenkins, U. S. Department of Agriculture, Bureau of Chemistry, Washington, D. C.
15. W. R. Austin, Tennessee Chemical Co., Nashville, Tenn.
16. F. S. Lagasse, University of Delaware, Agricultural Experiment Station, Newark, Del.
17. S. E. Asbury, College Station, Tex.
18. G. A. Shuey, Agricultural Experiment Station, Knoxville, Tenn.
19. C. H. Jones and G. F. Anderson, Agricultural Experiment Station, Burlington, Vt.
20. A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

DISCUSSION AND CONCLUSIONS.

A summary of the results, showing the averages obtained by each method by each collaborator, is given in the table. A study of these results reveals certain facts of importance. In the first place, the results obtained by the Breckenridge method on Sample No. 2, containing tankage, showed no consistent variation from those obtained by the official method. The total average results for each of these methods were practically identical. In Sample No. 1, which contained no organic material, the results from the two methods also checked closely, as would be expected. The tendency for the Breckenridge method to run slightly higher than the official method may be accounted for by the manner in which the aliquot was taken. For example, an 8 gram sample was used and made up to 200 cc., but on account of the space occupied by the material, an aliquot equivalent to 1 gram would actually contain slightly more than 1 gram. The error thus introduced, although nearly inappreciable, might account for the very slight increase in the results obtained by this method over those obtained by the official method. Although further work should be done with other fertilizers containing different organic materials before definite conclusions are drawn in regard to the Breckenridge modification, it is the opinion of the associate

TABLE 1.

Averages of collaborative results.

(Expressed as percentage of nitrogen.)

COLLABORATORS	SAMPLE NO 1.—$NaNO_3$, KCl, and acid phosphate. Theoretical value 3 88 %		SAMPLE NO. 2.—$NaNO_3$, KCl, tankage, and acid phosphate. Theoretical value 3.88 %		SAMPLE NO. 3.—$NaNO_3$, KCl, cyanamide, and acid phosphate. Theoretical value 1.94 %		SAMPLE NO. 4.—$NaNO_3$, KCl, urea, and acid phosphate. Theoretical value 1.94 %	
	Official method	Breckenridge modification	Official method	Breckenridge modification	Official method	Jones method	Official method	Jones method
1	3.78	3.80	4.15	4.04	2.52	2.34	2.18	2.16
2	3.95	3.95	3.63	3.77	3.23	2.48	2.27	2.03
3	4.08	3.91	3.75	3.96	3.00	1.92	2.55	2.08
4	4.01	3.97	3.77	3.71	3.16	1.81	2.33	1.79
5	3.92	4.06	4.06	4.13	2.95	2.36	2.24	2.09
6	3.98	3.98	3.82	3.80	3.25	2.22	2.56	1.99
7	4.02	4.07	4.11	4.14	3.58	2.58	2.39	2.35
8	4.06	3.83	3.94	3.78	3.13	1.94	2.26	2.21
9	3.91	3.70	3.91	3.89	2.60	2.20	2.48	1.98
10	4.05	4.03	4.04	3.98	3.46	2.49	2.44	1.96
11	3.97	4.04	4.14	3.97	3.52	2.51	2.44	1.91
12	4.00	3.96	4.07	3.98	3.48	2.22	2.44	1.87
13	4.03	4.08	4.18	4.18	3.60	2.37	2.38	1.83
14	3.99	4.05	4.11	4.12	3.23	2.11	2.40	1.66
15	4.17	4.49	4.23	4.51	3.36	1.86	2.44	2.05
16	3.92	3.98	3.66	3.59	2.78	1.92	2.29	1.80
17	3.78	4.04	4.06	4.04	2.72	2.18	2.46	1.21*
18	3.88	3.94	3.58	3.77	2.82	2.46	2.23	1.97*
19	4.00	....	3.96	.	3.40	2.13	2.28	2.04
20	3.93		3.92	....	3.63	2.26	2.31	1.96
Average	3.97	3.99	3.95	3.96	3.17	2.22	2.37	1.99

* Omitted from average.

referee that there is not enough variation in the two methods to warrant a change in the official method. Besides, the Breckenridge method does not eliminate the effects of soluble organic nitrogen, as was shown in last year's report.

The study of the Jones method for determining nitrate nitrogen in fertilizers containing cyanamide and urea yielded some pronounced and interesting results. The last four columns in the table give average results by this method in comparison with those by the official method. In Sample No. 3, which contained calcium cyanamide, the total average nitrate nitrogen content by the official method was 3.17 per cent, or 1.23 per cent above the theoretical value. By the Jones method the total average by all the collaborators was 2.22 per cent, or 0.28 per cent above the actual value. A number of the workers came much closer to the actual value by the Jones method than these average figures show. On Sample No. 4, which contained urea, the same condition holds true except that the variation between the two methods is not so great. The total average nitrate nitrogen content by the official method was 2.37 per cent, while the Jones method averaged 1.99 per cent, or 0.05 per cent over the actual value. The results of Collaborators 17 and 18 on Sample No. 4 by the Jones method were omitted from the average. As Collaborator 18 met with difficulties that caused variable results, it was thought best to omit them from the general average.

In the official method, when nitrate determinations are made, the hydrogen evolved from the iron and sulfuric acid acts to some extent on the organic materials and causes high results. In the proposed Jones method, the action of the hydrogen on these materials is offset by the use of a concentrated soda solution. In part B of the analysis the sulfuric acid and concentrated soda balance the effect on the organic materials of the iron, sulfuric acid, and soda treatment of part A. Thus, part B of the Jones method serves the purpose of obtaining a proper correction blank.

Although the collaborative results by the Jones method are not so close to the theoretical value as would be desirable, they certainly show a great improvement over the results obtained by the official method, especially when cyanamide is used. No doubt with further study the method will be improved.

Since this report was prepared results of analysis of the samples were received from the laboratories at Oppau through the courtesy of Kuttroff, Pickhardt & Co., makers of synthetic nitrogen products. The results on the Jones method were in close agreement with the results of the American collaborators, but with the official reduced iron method and the Breckenridge method their results ran low on Samples No. 1 and No. 2. Several other methods were tried out in addition to those specified, one of which was the Busch nitron method[1]. The German collaborators found this method applicable to the determination of nitrate nitrogen under any conditions, and whether or not cyanamide or urea was present. No doubt this method should be given consideration by the associate referee next year.

RECOMMENDATIONS[2].

It is recommended—

(1) That further work on the Breckenridge method be discontinued for the present.

(2) That the Jones method for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide and urea be further studied.

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[1] Treadwell and Hall Quantitative Analysis, Vol II, 1924, p. 391; Ber., 1905, 38: 861; Z. angew Chem., 1905, 13: 494.

[2] For report of Sub-committee A and action of the association, see *This Journal*, 1927, **10**. 65

A STUDY OF THE ALKALINE AND NEUTRAL PERMANGANATE METHODS AND COMPARISON OF RESULTS ON RAW MATERIALS AND FERTILIZER MIXTURES.

By H. C. MOORE and ROBERT WHITE (Armour Fertilizer Works, Chicago, Ill.).

INTRODUCTION.

The Jones alkaline permanganate method[1] and the Street neutral permanganate method[1] for the determination of nitrogen have been in use for many years and have been adopted as official by the A. O. A. C. They have been of much value in differentiating between organic nitrogenous materials of good quality and high availability and those of poor quality and low availability. These methods were originally intended for application to raw materials containing nitrogen in organic forms, either animal or vegetable, rather than to mixed fertilizers, because when they were devised organic materials furnished most of the nitrogen in fertilizer mixtures. Later, the methods were slightly modified for application to mixed fertilizers. In the fertilizer mixtures of the present day, a relatively small quantity of the nitrogen is derived from animal or vegetable sources.

Those that have had experience with these methods have noted that it seems strangely difficult to obtain even reasonably concordant results from several laboratories by either method. If portions of the same sample of an organic material or a fertilizer mixture containing such material are tested in several laboratories by the two methods, a range of results by the alkaline method, say from 45 to 65 per cent, or from 75 to 95 per cent by the neutral is not uncommon. While such discrepancies have frequently been observed, comparatively little attempt has been made to discover the cause and its correction.

It will be noted here that the above figures and those that follow apply to the percentage of activity of the water-insoluble organic nitrogen.

PURPOSE OF INVESTIGATION

The object of this paper is to set forth the results of a study of these methods by the writers, to point out certain details that must be most carefully followed, and to call attention to a number of causes of the discrepancies. It must be recalled that these methods are purely empirical and bear no exact relation to actual availability of the materials in the soil. Since they are empirical it is obvious that the methods must be followed exactly.

For the purpose of this study, the following samples of organic materials were selected:

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 12.

TABLE 1.

Collaborative results on determination of active nitrogen obtained by using official methods.

(Results are expressed in percentage.)

SAMPLES	ALKALINE PERMANGANATE METHOD			NEUTRAL PERMANGANATE METHOD		
	Official 1 mm	30-mesh	40-mesh	Official 1 mm	30-mesh	40-mesh
Chicago Blood (steam dried)						
Carteret	72.1	72.2	72.9	91.6	97.2	97.5
Atlanta	69.7	66.8	69.6	87.6	98.5	94.5
Nashville	74.2	.	75.2	93.3		93.3
4-12-4 (all ammonia from blood)						
Carteret	67.3	72.4	71.3	91.8	93.0	95.8
Atlanta	69 5	69.4	71.3	83.2	84 9	86.5
Nashville	70.6	.	71.4	91.5		97.1
4-12-4 (½% blood)*						
Carteret	61.5	67.7	68.3	76 9	77.4	78.3
Atlanta	50.7	57.2	58.6	87.7	88.6	85.7
Nashville	63.6	. .	57.2	80.4		82.5
Packing House Tankage						
Carteret	57.6	60 4	61.9	96.1	96.7	98.2
Atlanta	59.8	55.7	58.6	93.2	95.4	94.5
Nashville	69.8	70.5	71.2	. .	...	87.7
Tankage in 4-12-4 (2% tankage, 2% sulfate of ammonia)						
Carteret	64.6	65.4	65.1	89.8	92.3	93.3
Atlanta	55.1	55.4	56.7	89.3	89.2	89.2
Nashville	69.8	69.8	76.5	92.1	88.3	90.5
Tankage in 4-12-4 (1% tankage, 40 pounds of cyanamide, balance sulfate of ammonia)						
Carteret	65.9	67.1	67.8	84.7	89.4	86.2
Atlanta	58.6	62.1	56.3	82.8	87.8	85.1
Nashville	59.0	59.9	73.3	90.0	88.4	87.8
Nitrogenous Tankage						
Carteret	54.2	59.1	59.0	94.4	92.8	97.3
Atlanta	49.1	50.3	51.7	90.9	93.8	92.5
Nashville	55.4	59.2	61.1	91.4	91.5	83.6
4-12-4 (2% nitrogenous tankage, 2% sulfate of ammonia)						
Carteret	57.9	60.1	57.8	85.5	84.6	90 4
Atlanta	49.4	50.4	51.8	89.0	88.6	82.2
Nashville	56.2	58.7	62.2	89.5	90.1	89.9

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* Additional results by Carteret· 4-12-4 (½% blood, balance cyanamide, sulfate of ammonia, urea), 64.1; (½% blood, balance cyanamide, sulfate of ammonia, calcium nitrate, 61 3, (½% blood, balance cyanamide and Leunasalpeter) 60.0.

TABLE 1—Continued.

Collaborative results on determination of active nitrogen obtained by using official methods.

(Results are expressed in percentage.)

SAMPLES	ALKALINE PERMANGANATE METHOD			NEUTRAL PERMANGANATE METHOD		
	Official 1 mm.	30-mesh	40-mesh	Official 1 mm.	30-mesh	40-mesh
4-12-4 (1% nitrogenous tankage, 40 pounds cyanamide, sulfate of ammonia)						
Carteret	58.2	61.3	58.7	77.7	68.8	72.0
Atlanta	50.6	53.0	55.4	69.8	78.3	76.5
Nashville	52.8	57.8	60.5	85.6	83.4	84.9
4-12-4 (½% nitrogenous tankage, 40 pounds of cyanamide, soda, sulfate of ammonia)						
Carteret	60.0	62.5	63.2	72.5	60.0	60.5
Atlanta	49.0	52.0	52.0	72.5	78.0	76.0
Nashville	58.2	61.4	48.8	72.9	72.8	72.1
2-12-2 (½% nitrogenous tankage, sulfate of ammonia)						
Carteret	54.5	64.0	52.2	45.4	60.0	60.9
Atlanta	50.0	59.1	59.1	54.2	54.5	50.0
Nashville	57.1	57.1	59.1	68.2	70.4	66.7
Castor Pomace						
Carteret	58.9	56.2	52.1	92.9	92.7	92.5
Atlanta	47.0	45.6	45.9	92.0	92.0	91.6
Nashville	58.6	63.4	64.0	91.3	91.4	89.3
4-12-4 (1% nitrogenous tankage, 200 pounds of castor = 0.61%, balance sulfate of ammonia)						
Carteret	57.9	57.5	52.3	91.0	94.0	89.1
Atlanta	50.4	56.0	59.2	87.8	89.5	90.4
Nashville	....	....	61.5	85.7	89.4	89.6
Garbage Tankage						
Carteret	30.2	29.6	25.3	75.6	76.4	82.2
Atlanta	23.5	20.4	23.2	72.2	72.3	73.8
Nashville	29.2	31.9	33.2	76.3	77.2	66.7
4-12-4 (1% nitrogenous tankage, 200 pounds of garbage = 0.35%. Balance sulfate of ammonia)						
Carteret	45.5	46.5	45.9	76.2	81.2	79.4
Atlanta	42.1	46.8	42.9	85.0	81.2	77.1
Nashville	45.1	50.0	53.8	79.5	80.7	81.1

	AMMONIA* *per cent*	PHOSPHORIC ACID *per cent*
Dried blood (steam dried)	17	
Packing house tankage	8.5	10
Nitrogenous tankage	9	
Garbage tankage	3.5	
Castor pomace	6	

* Approximate.

In addition, a number of samples of complete fertilizer mixtures were prepared, in which the same materials were used as sources of water-insoluble organic nitrogen. These mixtures were not abnormal; they were in line with ordinary fertilizer manufacturing practice. The samples were carefully prepared by White, in the company's laboratory at Carteret, N. J., and portions of each sample were sent to two of the other laboratory analysts, R. D. Caldwell, Atlanta, and W. R. Austin, Nashville.

The instructions given were to follow exactly the two official methods. All the samples were prepared to pass a 1 mm. circular mesh screen, as specified in the methods. Portions of each sample were also ground to pass a 30-mesh and a 40-mesh screen, respectively, in order that the effect of finer grinding of the samples might be studied. The following tabulation is a summary of the results reported by the three laboratories on these samples. Altogether about three thousand determinations were made.

In Table 2 appears a summary of the results from the foregoing tables obtained in the Carteret laboratory.

TABLE 2.

Results on activity of nitrogen (water insoluble) obtained in Carteret Laboratory.

	NEUTRAL *per cent*	ALKALINE *per cent*
Blood series:		
Dried blood alone	92	72
4-12-4 fertilizer (4 per cent ammonia from blood)	92	67
4-12-4 fertilizer (½ per cent ammonia from blood, 40 pounds cyanamide, balance sulfate of ammonia and nitrate)	77	62
4-12-4 fertilizer (same, but no cyanamide)	84	63
Packing house tankage series:		
Packing house tankage alone	96	58
4-12-4 fertilizer (2 per cent ammonia from tankage, 2 per cent ammonia from sulfate of ammonia)	90	65
4-12-4 fertilizer (1 per cent ammonia from tankage, 40 pounds of cyanamide per ton, sulfate of ammonia)	85	66
Nitrogenous tankage series:		
Nitrogenous tankage alone	94	54
4-12-4 fertilizer (2 per cent ammonia from tankage, 2 per cent ammonia from sulfate of ammonia)	85	58
4-12-4 fertilizer (1 per cent ammonia from tankage, 40 pounds of cyanamide per ton, sulfate of ammonia, nitrate)	78	58
4-12-4 fertilizer (½ per cent ammonia from tankage, 40 pounds of cyanamide per ton, sulfate of ammonia)	72	60
4-12-4 fertilizer (same as above but no cyanamide)	67	58
2-12-2 fertilizer (½ per cent ammonia from tankage, sulfate of ammonia)	45	55

The results in Table 2 obtained in the Carteret laboratory were segregated, partly for convenience and partly because the results from this laboratory were on the whole more uniformly consistent, owing, no doubt, to the greater experience and familiarity of the analysts with the methods, particularly the alkaline method. These results indicate that the nitrogen activity found in the samples of mixed fertilizers is not the same as that found on the raw materials used in these mixtures. In the case of the sample of blood, the activity by the neutral method drops from 92 per cent in the raw material to as low as 77 per cent in the mixtures, while by the alkaline method it drops from 72 per cent to 62 per cent. In the case of packing house tankage, likewise, the activity drops from 96 per cent to 85 per cent by the neutral method, but it increases from 58 per cent to 66 per cent by the alkaline method. In the case of the nitrogenous tankage the activity drops from 94 per cent to as low as 45 per cent by the neutral method, whereas it increases slightly by the alkaline method.

In the case of a sample of blood received from South America, the Carteret laboratory found about the same activity for the blood in mixtures as for the raw material itself, as shown in Table 3.

The samples constituted a preliminary group to obtain mixtures to show differences in activity of the raw material and mixed goods and were not used for further work.

TABLE 3.

Results obtained in the Carteret Laboratory on a sample of South American blood and mixtures made therefrom.

DETERMINATIONS	A	B	C	D	E
	Blood	*lbs.* Blood......250 S/A.......160 A P......1430 M/Pot.....160	*lbs* Cyanamide..40 Blood......250 A. P......1430 M/Pot. .. 160 S/A...... 120 4—12—4	*lbs.* Cyanamide. 75 Blood.... .125 A. P.. 1480 M/Pot.....160 S/A... 160	*lbs* No. C .. 1120 S/A . . 140 A. P. .. 670 M/Pot. .70
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Water-insoluble ammonia...........	16.80	2.06	2.04	1.12	1.14
Alkaline active (water-insoluble ammonia).	12.42	1.46	1.46	.80	.82
Alkaline activity (water-insoluble ammonia).	73.9	70.9	71.6	71.4	71.9
Neutral activity (water-insoluble ammonia)..	93.8	95.1	95.1	91.1	92.1

It has been observed from the results given in Table 3 and from other results that the activity values of an organic material in mixtures may or may not correspond with the value for the raw material alone. In the special samples that have been sent out by E. W. Magruder for check analysis the same condition has been observed. In the case of a sample of blood and of a fertilizer mixture made with this blood, the average of nitrogen activity results from all laboratories was about the same for each sample.

More recently Magruder has sent a sample of dried fish, called his sample No. 10, and later his sample No. 11, marked 4-8-6 fertilizer, made on the following formula:

	pounds
Acid phosphate	940
Dried fish (Sample No. 10 above)	200
Sulfate of ammonia	200
20 per cent manure salts	620

The results for nitrogen activity have been summarized as follows:

NUMBER OF LABORATORIES REPORTING	SAMPLE NO	NITROGEN ACTIVITY	
		ALKALINE	NEUTRAL
		per cent	*per cent*
21	10	74.0	95.8
21	11	67.6	94.4

It was observed from a study of the tabulations submitted that the personnel in the two lists did not exactly correspond. However, as it was found that thirteen of these collaborators reported on both samples 10 and 11, the following tabulation, differing but slightly from the above, was prepared to show the comparison for the same thirteen collaborators.

NUMBER OF LABORATORIES REPORTING	SAMPLE NO.	NITROGEN ACTIVITY	
		ALKALINE	NEUTRAL
		per cent	*per cent*
13	10	74.5	96.3
13	11	69.3	94.9

It appears, therefore, that some materials show about the same value in mixed goods as alone; others do not, but whether this is due to the material itself or to the effect of the other materials in the mixture is not known.

The mixtures containing garbage tankage showed somewhat lower activity than that calculated from the two materials used alone, while in the case of castor pomace but little change was noted. In the case of these materials, however, conclusions are difficult because they were not the only organic matter in the mixture.

Because the results given in the tables, particularly Tables 1 and 2, show such variances, a study of the different stages of the methods was undertaken, such as:

Fineness of the sample,
Thoroughness of the preliminary water washing,
Effect of paraffin in the alkaline method,
Length of initial digestion period by the alkaline method,
Rate of distillation by the alkaline method,

Amount of distillate by the alkaline method,
Volume of final washing by the neutral method.

Before this final study of the details was begun, the results were submitted to C. H. Jones, author of the alkaline method, in order that the benefit of his experience might be available. His suggestions enabled the writers to locate some of the causes of the discrepancies. It was discovered that the present published form of the official alkaline method differs in important details from the original method. Apparently certain details have been deleted. For example, the original method did not mention the addition of paraffin and did provide for a period of distillation of about one hour. These details and others are important, as will be shown in the following directions summarized as the result of this study of the methods.

FINENESS OF SAMPLE.

It is important that a uniform fineness of samples be used for nitrogen activity determinations; otherwise concordant results are impossible. If the sample is ground finer than a 1 mm. circular mesh, somewhat higher results are obtained by the neutral method in the case of high-grade materials, while by the alkaline method the results on the finer samples are sometimes lower than on the coarser. These lower results may be explained (in the alkaline method) by the fact that the finer sample offers more surface to the action of the permanganate, which is usually entirely and more quickly exhausted. It is well known in the case of low-grade materials, that if the quantity of permanganate is increased, the activity results are higher, and the more the quantity of permanganate is reduced, the lower the results will be.

WATER-INSOLUBLE NITROGEN.

It has been observed frequently that when several analysts collaborate on a given sample, they usually differ much more in their results for water-insoluble nitrogen than for total nitrogen; they probably would not all take the same quantity of sample calculated to furnish 50 mg. of water-insoluble nitrogen, and therefore uniform results could not be expected. Unless the washed sample actually contains 50 mg. of nitrogen, the calculations will not be correct. A study of the water washing was made, and it was found that in the case of high-grade materials, such as blood, usually less than 50 mg. of nitrogen remained, while in the case of mixtures, the opposite condition prevailed. When actually determining the nitrogen present in a number of washed samples calculated to furnish 50 mg. of water-insoluble nitrogen, anywhere from 47 to 57 mg. remained, as shown in the following tabulation:

SAMPLE	QUANTITY OF SAMPLE TAKEN ESTIMATED TO FURNISH 50 MG OF INSOLUBLE NITROGEN		ACTUAL QUANTITY OF NITROGEN FOUND AFTER WASHING
	gram		*mg.*
Blood	0.362		48.0
Packing house tankage	0.917		48.7
Nitrogenous tankage	0.944		47.5
Mixed goods (½ per cent ammonia from blood, balance sulfate of ammonia and nitrate)	15.2	(250 cc.)	57.4
		(500 cc.)	56.3
		(750 cc.)	55.0

Obviously the washing with water is one of the most important steps in either of these methods.

Doubtless one reason why the results in the case of mixed goods did not correspond with results on the raw material used, is the fact that in mixed fertilizers the washing is not done with water, but it is done with a solution in which the various other fertilizer materials are more or less soluble.

After noting this fact and taking steps to insure having actually 50 mg. of nitrogen present in the washed residue, the several laboratories were able to agree more closely. This will be noted in a subsequent tabulation, in which the effect of a uniform procedure throughout is also shown.

PRELIMINARY DIGESTION IN ALKALINE PERMANGANATE METHOD.

The official method prescribes that after the washed sample is dried, it be transferred with 20 cc. of water and 100 cc. of alkaline permanganate solution to a Kjeldahl flask. After connecting the flask to a condenser, digest the sample slowly for at least 30 minutes below the distillation point, etc. It was found that varying this preliminary digestion period made but little difference, providing the distillation period was not too short, that is not less than one hour; so far as the preliminary digestion period itself is concerned, thirty minutes seems sufficient, except to prevent subsequent frothing in the case of such samples as may require a longer period.

DISTILLATION ALKALINE PERMANGANATE METHOD.

The official method states: "* * * after all danger from frothing has passed, distil until 95 cc. of the distillate is obtained. Titrate with standard alkali, using cochineal or methyl red indicator". The results obtained by the writers have shown a wider discrepancy due to irregularity in the distillation period than in any other stage of the alkaline method. It developed that each of the three laboratories was distilling the 95 cc. at different rates. Atlanta made the distillation in about 30 minutes, Carteret in about 60 minutes, and Nashville in about 90 minutes. The Atlanta results were always low and the Nashville results generally a little higher than Carteret. Jones advises that in his original method a distillation period of one hour was recommended.

The following tabulation shows the results on nitrogen activity obtained in one of the laboratories following a 30 minute preliminary digestion period, by varying the rate of distillation from 30 minutes to 90 minutes:

SAMPLE	DISTILLATION PERIOD 30 MIN. *per cent*	DISTILLATION PERIOD 90 MIN. *per cent*
Blood	68	73
Packing house tankage	58	70
Nitrogenous tankage	56	62
Castor pomace	55	58
Garbage tankage	27	31

And the following tabulation shows the results from another laboratory obtained after a preliminary digestion of 30 minutes and distilling 95 cc. in 30, 45, and 60 minutes:

SAMPLE	DISTILLATION PERIOD 30 MIN. *per cent*	DISTILLATION PERIOD 45 MIN. *per cent*	DISTILLATION PERIOD 60 MIN. *per cent*
Chicago blood	68	71	76
Packing house tankage	59	61	66
Nitrogenous tankage	49	53	56
Castor pomace	41	45	51
Garbage tankage	19	21	25
Mixture No. 1	65	69	67
2	33	41	48
3	35	41	45
4	38	44	49
5	42	47	51
6	61	68	70
7	62	68	73
10	46	50	52
13	57	61	66
16	33	42	50

It will be observed that the official method, which is purely empirical, should be more specific on this point, and that such details should not be left to individual interpretation.

COLLECTING THE 95 cc. DISTILLATE BY THE ALKALINE METHOD.

It is important that the distillate be exactly 95 cc., as more or less distillate affects the final results appreciably. Lower results are obtained if less than 95 cc. is collected and higher results if more than 95 cc. The following tabulation shows the effect on the percentage of alkaline activity of continuing the distillation up to 110 cc., which it is possible to collect from the quantity of solution added to the distilling flask:

SAMPLE	95 cc.	110 cc.
Packing house tankage	59	65
Garbage tankage	26	31
Nitrogenous tankage	50	53
Castor pomace	51	55

It should be noted that it is important to add exactly the 20 cc. of water, together with 100 cc. of alkaline permanganate solution. This

20 cc. should be measured with a pipet or other accurate measuring instrument. It is even advisable to remove the dried residue from the paper by scraping it into the flask, finally brushing any remaining on the paper rather than attempt to wash it in with the 20 cc. of water. It is advisable to collect the distillate in a slender tube or bottle so graduated or marked that the quantity can be accurately measured.

The experiments of the writers have shown that if additional water is added to the distillation flask and the distillation continued, considerable ammonia continues to come off, an additional 100 cc. of water added in two 50 cc. portions having been found to increase the activity results from 5 to 10 per cent. However, if all this additional water were added in the first place, along with the 20 cc. of water and 100 cc. of alkaline permanganate solution, lower results would be obtained than when the official method is followed. This is probably due to the different concentration of the solution. In this connection the following experiment was tried: The results in column A of the following tabulation were determined by distilling 95 cc. as in the official method; under column B the procedure was the same as under A, except that when the 95 cc. had been distilled, two separate portions of 50 cc. of water were added to the distilling flask and the two distillates obtained, the results being added to A. The results under column C were obtained by adding to the distilling flask in the first place 100 cc. of water, in addition to the 20 cc. of water and 100 cc. of permanganate.

SAMPLE	A OFFICIAL	B +100 CC. OF WATER	C +100 CC. OF WATER AT FIRST
	per cent	*per cent*	*per cent*
Blood	73	81	67
Packing house tankage	62	71	58
Nitrogenous tankage	59	64	50
Castor pomace	52	65	46
Garbage	25	33	22

The results in this tabulation are not important, except that they show the necessity of rigidly following the details set forth. It has been observed that the temperature in the distilling flask gradually rises to about 140°C. at the end of the distillation. It is apparent that the effect of temperature and concentration in this hydrolysis, during which ammonia is formed, is important and that uniform results are possible only under uniform procedure.

USE OF PARAFFIN IN ALKALINE METHOD.

The official method prescribes the addition of a small piece of paraffin. This recommendation did not appear in the original Jones method. The tests reported in this paper have indicated that from 1 to 1½ grams of permanganate can be reduced in a blank test by varying the amount of paraffin added. This reduction is not due entirely to the amount of paraffin; it is partly due to the fertilizer materials present and possibly to the boiling in alkaline solution. A series of blank tests was made, 100 cc. of alkaline permanganate solution with 20 cc. of water being taken and paraffin in different amounts being added. After distilling 95 cc. of the solution, the excess permanganate remaining was determined as follows:

PARAFFIN USED	DISTILLED	PERMANGANATE REDUCED
	cc.	*grams*
0 gram	95	0.50
pea size	95	1.06
pea size	75	1.25
pea size	50	.75
pea size	25	.53
5 grams	95	1.70

It appears that paraffin has an important effect on the permanganate, and it seems desirable to clarify the method in this respect. The use of a few perforated glass beads is sufficient to prevent frothing.

NEUTRAL PERMANGANATE METHOD.

The details of the neutral permanganate method are probably more uniformly controlled, although the results are but little more concordant than by the alkaline method. The important point in this method seems to be the thorough preliminary washing with water. It might appear that this is unimportant, or less important than in case of the alkaline method, on the assumption that in case the nitrogen is not completely washed out in the first place with water, it would be after the permanganate digestion.

Some parallel tests were made in which the same quantities of several samples were taken. In one set the preliminary washing with water was made, in the other the sample was used unwashed. The quantity of inactive ammonia remaining finally in both sets after the permanganate treatment and washing was as follows:

SAMPLE	OFFICIAL	UNWASHED BEFORE DIGESTION
	per cent	*per cent*
Packing house tankage .	0.47	0.60
Nitrogenous tankage. . .	0.61	0.85
Garbage tankage.	0.82	1.53
Castor pomace.	0.47	0.63

It would appear from the results given that in case the water-soluble organic nitrogen is not completely washed out, some of it may be rendered insoluble in the digestion period and low results produced therefrom. The results given here do not indicate that it is necessary to wash in excess of 400 cc. after the permanganate digestion. A number of determinations with washing to 600 cc. were made, the results being practically the same. Why so much discrepancy exists among analysts using the neutral method has not appeared from this work, unless it is due to the failure to have the sample thoroughly washed with water and to have exactly 50 mg. in the washed residue. The greater portion of the writers' time has been devoted to a study of the alkaline method, inasmuch as this method is generally used by fertilizer control officials.

MISCELLANEOUS DETERMINATIONS.

The many miscellaneous investigations made in connection with this work are perhaps of little value when considered in a detached way, but some of the results throw light generally upon the methods, and especially upon the importance of adhering strictly to the details set forth.

It is well known that if the amount of permanganate is increased, in the case of garbage tankage, the percentage of nitrogen activity would likewise increase, especially with the alkaline method. It has been found, however, that even the use of 100 cc. of alkaline permanganate in this connection usually gives a result that is higher than the actual availability of the material in the soil. It is interesting to note, however, that when a sample of blood is diluted with nitrogen-free filter paper pulp, so that the mixture contains about the same percentage of ammonia as garbage, and a sample of this mixture equivalent to 50 mg. of nitrogen is taken for treatment with alkaline permanganate, the apparent activity is about the same as for garbage, showing the effect of organic matter on the permanganate used. This would indicate that if a filler or other material containing organic matter were used in fertilizer manufacture, the action of this organic matter on the permanganate would cause apparently low results. The results by the neutral method, however, on a paper-blood mixture are but little lower than for the blood alone. The same was found true in the case of a sample of nitrogenous tankage. When acid phosphate was used in place of the paper, with blood and tankage, no appreciable change was noted.

Blank tests made with varying quantities of filler, sand and phosphate rock screenings with acid phosphate, sulfate of ammonia and potash salts, seemed to have but little more effect on the permanganate used by either method. The effect in the case of the alkaline method, in which 25 grams of such mixture was used, amounts to but little more than the effect of the paraffin alone. These tests were made with paraffin, and up to 1.15 grams of permanganate was thus reduced. By the neutral method, 25 grams of sample caused the reduction of about ¼ gram of permanganate. The following tabulation summarizes these results:

Sample C = 160 pounds of sulfate of ammonia, 1200 pounds of 20 per cent acid phosphate, 200 pounds of manure salts, 440 pounds of sand.

	PERMANGANATE REDUCED *gram*
Alkaline method—5 grams of above sample washed and dried, (official) remove from paper and distil off 95 cc., using paraffin	0.92
Alkaline method—25 grams of above sample with official procedure	0.94
Neutral method—5 grams of above sample with official procedure	0.13
Neutral method—25 grams of above sample with official procedure	0.24

	PERMANGANATE REDUCED *gram*
Sample N = 160 pounds of sulfate of ammonia, 1200 pounds of 20 per cent acid phosphate, 200 pounds of manure salts, 440 pounds of rock dust, 45 per cent of bone phosphate of lime.	
Alkaline method—5 grams of above sample with official procedure.	0.85
Alkaline method—25 grams of above sample with official procedure......	1.14
Neutral method—5 grams of above sample with official procedure.	0.20
Neutral method—25 grams of above sample with official procedure.......	0.28

Some interesting results were obtained on a sample of Peruvian guano, which showed 37 per cent activity by the alkaline and 97 per cent by the neutral method. Using double the amount of permanganate, that is double the quantity of actual potassium permanganate in the same volume of solution, in the alkaline method, the percentage of activity was 27 per cent. These anomalous results are apparently due to the fact that Peruvian guano contains uric acid, which is difficultly soluble in water and which is oxidized by the permanganate solution, probably to urea, which is but slightly converted into ammonia by distilling with alkaline permanganate.

When, however, a sample of Peruvian guano containing 50 mg. of insoluble nitrogen was treated with ¼ gram of permanganate, digested for 30 minutes, after which the permanganate was reduced with ferrous sulfate, ¼ gram of urease added (which converts urea quantitatively to ammonia), and after standing 30 minutes was distilled with caustic soda, 67 per cent of the ammonia was thus recovered.

It would seem from the above that reasonable allowances must be made in the case of mixed fertilizers containing relatively small quantities of organic nitrogen and large quantities of other fertilizer materials, or else some effort should be made to make a separation of the organic materials, possibly by carbon tetrachloride, from the remainder of the sample, as it is obvious that when as much as 15 grams of a sample is required to furnish 50 mg. of water-insoluble nitrogen, complete removal of the water-soluble with water is difficult, if not impossible.

The results of this work would indicate that the alkaline permanganate method should be applied about as follows:

ALKALINE METHOD.

Place a quantity of the sample containing the equivalent of 50 mg. of water-insoluble organic nitrogen on a filter paper and wash thoroughly with water until the filtrate measures 250 cc. In the case of mixed fertilizers, where the sample amounts to 4 grams or over, wash several times by decantation in the beaker, finally transferring to a filter and then washing to 250 cc. Occasional tests should be run on some of the washed samples to be sure that 50 mg. of insoluble nitrogen is being used in the determination.

In the case of raw materials to which 2 grams of phosphate rock is added, it would be better to mix the sample in the beaker in a wet way rather than to grind in a mortar, which would have the effect of using a sample finer than the official mesh.

It is only necessary to use the ether washing where much oil or fat is present.

Dry paper and contents below 80°C., transfer sample from paper to a Kjeldahl flask, taking care not to scrape the paper to include paper pulp, and add exactly 20 cc. of water from a pipet or buret, which may be added to facilitate transferring the sample to the flask; then add exactly 100 cc. of alkaline permanganate solution. Do not add paraffin, but add a few perforated glass beads, connect the flask to an upright condenser, and digest below the boiling point for 30 minutes, or longer if found necessary to prevent foaming. Finally raise the temperature and distil over exactly 95 cc. collected in a slender bottle or graduate containing standard acid into which the delivery tube dips. Distil at a uniform rate so that 95 cc. is collected in about 1 hour, not less, rather a little longer. It is well to test the last few drops of distillate and occasionally to make test of rinsing out the tubes, to be sure that all the ammonia is recovered. (Clean tubes should not hold droplets of weak ammonia.)

NEUTRAL METHOD.

Take a sample equivalent to exactly 50 mg. of water-insoluble nitrogen, as under the alkaline procedure, wash thoroughly as described under the latter, and finally transfer the insoluble residue with 25 cc. of tepid water into a 300 cc. beaker and add 1 gram of sodium carbonate; mix and add 100 cc. of 2 per cent permanganate solution, etc., proceeding exactly as described in the official methods, finally washing the residue on the paper until the filtrate amounts to 400 cc.

GENERAL.

It is well to emphasize once more that empirical methods are being considered, and they must be followed exactly if any reasonable agreement is to be obtained. The following brief tabulation shows the agreement among the three laboratories mentioned previously, using the alkaline method, before and after observing all the precautions outlined above:

SAMPLE	ATLANTA		CARTERET		NASHVILLE	
	First work	Final	First Work	Final	First Work	Final
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blood	70	75	72	75	74	75
Packing house tankage	60	65	58	67	70	69
Nitrogenous tankage. . .	49	59	54	62	56	61

While this agreement is not entirely satisfactory, it does show plainly that more uniform results will be obtained under uniform procedure.

It is not the purpose of the writers to criticize or condemn either of the permanganate methods. They have been found useful for many years, and when both are used in case of doubtful samples, they usually determine between materials of good quality and those that are inferior.

It is important, however, that the official methods be made more explicit, especially in the case of the alkaline method. Inasmuch as some organic materials seem to have different activity values when used in mixtures and when used alone, certain reasonable allowances should be made in the application and interpretation of the methods and the standards that are adopted; particularly should this be true in the case of samples where the quantity of organic material is relatively small.

It would, therefore, seem unwise, until a more careful study has been made of the methods and their application to various mixtures, to increase the present standards, which the Committee on Definitions of Terms and Interpretation of Results on Fertilizers of the A. O. A. C. has recommended, namely 50 per cent by the alkaline method and 80 per cent by the neutral method.

This paper is submitted in the hope that it may be of help to others and tend in some measure to clarify the methods and bring about a more uniform interpretation.

ANALYSIS OF CALCIUM NITRATE[1].

By J. M. McCANDLESS and J. Q. BURTON (McCandless Laboratory, Atlanta, Ga.).

The problem of analyzing calcium nitrate BASF, a synthetic product made in Germany, has recently been presented to the writers. Furthermore, it was requested that it be done by the official method in daily use in the State laboratories. The difficulty that this work presents is due entirely to the extremely hygroscopic nature of this salt. Those that have tried it will agree that it is impossible to weigh the portions required for analysis in the usual way with any degree of accuracy. Especially is this the case in humid weather, because the salt absorbs water with such rapidity that it is impossible to obtain an accurate balance even when weighing as fast as possible.

Under these circumstances it is considered necessary to describe in some detail the method of analysis finally adopted, which is as follows:

PREPARATION OF THE SAMPLE.

Before the sample presented for analysis is opened, make ready a large dry mortar and pestle, and a bottle with rubber stopper for the laboratory sample. Crush any lumps in the sample, but do not attempt to pass it through a sieve. Mix the material quickly in the mortar with a spatula and transfer it to the rubber stoppered bottle without delay. (The experience of the writers is that the material is so uniform in composition that it is only necessary to crush the lumps.)

DETERMINATION.

Place approximately 15 grams of the sample in a counterpoised weighing dish with close fitting cover, obtain the net weight of the calcium nitrate, wash into a liter flask, and make to the mark. First determine the nitrogen in this solution by the official Kjeldahl-Gunning method modified for nitrates, as follows: Transfer 25 cc. of the solution to a Kjeldahl flask and add 0.4 gram of sodium carbonate. (This converts the calcium nitrate into sodium nitrate and calcium carbonate and insures the expulsion of the small percentage of ammonia always present in this material in the form of ammonium nitrate.) Evaporate the solution carefully in the flask over a small flame to a small volume and then carry to dryness by revolving the flask over the wire gauze so as to spread it out over a large surface. (The addition of about half a gram of recently ignited talc at this stage helps materially in obtaining a dry residue.) Add the usual salicylic-sulfuric acid mixture, bring into thorough contact with the dry residue, and proceed with the analysis in the usual way. Distil another aliquot of the solution with caustic soda and add the nitrogen so obtained to that found by the digestion of the dry residue.

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[1] Presented by A. M. Smith.

To determine the degree of accuracy secured, known aliquots of c. p. sodium nitrate were subjected to the same procedure. The following results, expressed as percentage of nitrogen, were obtained:

CALCIUM NITRATE	SODIUM NITRATE C P
15.40	16.40
15.44	16.48
15.45	16.44
15.49	16.40
15.47	
15.50	

THE REDUCED IRON METHOD.

The reduced iron method would seem to be the natural and easy method to use in this case, and it would be used by the writers if they had ever been able to get theoretical results on a c. p. sodium nitrate when a deduction for the nitrogen contained in the reduced iron and the other reagents was made.

In the reduced iron purchased from one well-known maker, a blank amounting to more than a half of one per cent of nitrogen was found, and with the best iron powder purchasable, the blank amounted to 0.12 per cent when 0.35 gram of nitrate was used. After deducting this blank, the following results, expressed as percentage of nitrogen, were obtained by the writers by the reduced iron method on c. p. sodium nitrate and on a sample of calcium nitrate:

CALCIUM NITRATE	SODIUM NITRATE C P
15.24	16.24
15.30	16.32
	16.32
	16.18 (using 0.7 gram)

Considering these results, the writers were at a loss to understand why this method should reduce practically all the nitrate to ammonia, but allow a small percentage to escape. This loss seems to be due to the several reactions that occur when the sulfuric acid is added to the mixture, among which are the following:

(1) $2NaNO_3 + H_2SO_4 = Na_2SO_4 + 2\ (HNO_3)$

(2) $3Fe^{++} + NO_3 + 4H = 3Fe^{+++} + NO + 2H_2O.$

Some of the nitric oxide is carried along with the escaping hydrogen, passes out of the flask, and is lost. It may be absorbed and recovered.

In its passage through the flask and the absorbing solution the following reaction occurs:

$$2NO + H_2O + 3O = 2HNO_3.$$

The qualitative test with brucine applied to some of the absorbing liquid shows the presence of nitric acid. Doubtless the method may be so modified as to absorb or arrest all the escaping nitrogen and determine it either by titration or by returning the absorbing solution to the main body of the liquid in the flask, adding more iron, and completing the reduction.

The unusually early meeting of the A. O. A. C. prevented the writers from carrying out the experiments necessary to show the proper apparatus and modifications needed to make the reduced iron method as accurate as any other. It was found that the modified Kjeldahl-Gunning method as previously described gave the best results on calcium nitrate.

It is suggested that the Associate Referee on Nitrogen study these methods during the coming year. It is also suggested that the nitron method[1] and the Arnd method[2] for nitrates, requiring the Arnd alloy, be investigated and studied. Both methods are being used successfully abroad.

DETERMINATION OF MOISTURE IN CALCIUM NITRATE.

The determination of the water in a sample of calcium nitrate also presents peculiar difficulties. In a case of dispute as to the real content of nitrogen in a shipment of this material, where one sample contains more water than the other, it is important that the water in each be determined accurately. Pure calcium nitrate contains 4 molecules of water of crystallization, and at 130°C., the official temperature for drying the salt, some of this water is expelled and no results in satisfactory agreement can be obtained. The writers next tried the Sterling-Bidwell method[3] for moisture, using toluene (methyl benzene) boiling at 110°C. as the reagent for carrying over the water, and measuring it in the Sterling-Bidwell apparatus. By using this method on one sample and by boiling for 5 hours, 10.61 and 10.63 per cent of water was obtained, but on the same sample the percentage increased to 13.81 per cent when boiling was continued for 7 hours.

Using xylene (di-methyl benzene) boiling at 139°C., the same reagent used in Germany, and boiling for 6 hours, the percentage of water obtained in the same sample was 19.16 per cent. An examination of the cork in the boiling flask, however, showed slight decomposition after this prolonged exposure to the temperature of the boiling reagent.

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[1] Scott. Standard Methods of Chemical Analysis, 1917, p. 296; M. Busch. *Ber.*, 1905, 38: 861.
[2] Method used in laboratory of I. G. Farben Industrie, Oppau, Germany.
[3] *This Journal*, 1925, 8: 296.

Probably a mixture of toluene and xylene with a slight lowering of the boiling point would give good results, but no opportunity to test such a mixture has been afforded.

As an alternative, W. E. Simpson of this laboratory suggested and worked out the following chemical method for determining moisture:

METHOD FOR MOISTURE IN CALCIUM NITRATE.

Weigh approximately 10 grams of the calcium nitrate in a weighing dish. Wash into a 250 cc. flask with distilled water, dissolve, and make to mark. Weigh sufficient sodium carbonate to combine with the calcium nitrate (using the factor 0.646), dissolve in water in a 250 cc. flask, and make to mark Pipet 10 cc. of each solution into the same weighed dish. Evaporate on the water bath to apparent dryness, then dry in the oven for 3 hours at 130°C. Cool in a desiccator and weigh.

Calculate the combined weights of the salts used minus the ammonium carbonate driven off (the weight of ammonia multiplied by the factor 2.76). The difference between this figure and the weight of the dried residue is the amount of moisture given up by the calcium nitrate, the sodium carbonate used being dry. Having the quantity of calcium nitrate worked on and the loss in weight due to moisture, the percentage of moisture is easily calculated.

Using this method, the writers found in one of the samples tested, moisture 17.24 per cent, duplicate 17.28 per cent, and in another sample, moisture 12.66 per cent, duplicate 12.51 per cent.

REPORT ON POTASH[1].

By A. P. KERR (Agricultural Experiment Station, Baton Rouge, La.), *Associate Referee.*

Several methods were tried to eliminate the phosphoric acid in the water solution of potash, as recommended by this association during its meeting in 1925. The idea of making the water-soluble phosphoric acid insoluble by the addition of calcium carbonate, as recommended by G. S. Fraps[2], seems to be promising. It is recommended that this method be studied next year with the modifications offered by L. D. Haigh in a paper published in this number of *The Journal.*

Regarding the work of determining chlorine in commercial fertilizer, it may be stated that three methods have been tried during this year, and some progress along this line has been made.

RECOMMENDATIONS[3].

It is recommended—

(1) That a study be made of the use of calcium carbonate in preparing the solution for potash.

(2) That work on a method for chlorine in fertilizer be continued.

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[1] Presented by G. S. Fraps.
[2] *This Journal*, 1926, **9**: 192.
[3] For report of Sub-committee A and action of the association, see *This Journal*, 1926, **10**: 65.

A SUGGESTED MODIFICATION OF THE OFFICIAL METHOD FOR POTASH IN MIXED FERTILIZERS.

By L. D. Haigh (Agricultural Experiment Station, Columbia, Mo.).

The experience of fertilizer chemists indicates that the official method for determining the potash in mixed fertilizers is producing irregular results, mostly lower than the true percentage. Changes in the nature of the materials used in the manufacture of these fertilizers are probably accountable for the increasing difficulties in obtaining a true result with this method.

It has been pointed out by Kerr[1], Associate Referee on Potash, and by Bible[1] that the water-soluble phosphoric acid in the fertilizer washings is the cause of low results in the potash determination. The suggestion has been made that this be removed by the addition of magnesium chloride solution. The results of using this solution are, in many cases, quite satisfactory, but some difficulties have been experienced because the addition of an excess of it is liable to give too high results for potash. The use of magnesium chloride solution is reported quite fully by Kerr, however, and need not be discussed here.

A suggestion for the removal of water-soluble phosphoric acid in the potash determination is found in a method for potash presented by G. S. Fraps of this association[2]. The first part of the directions for this method reads as follows:

> Weigh 2.425 grams of sample into a beaker, add 2 grams of carbonate of lime and 25 cc. of water, and let stand an hour, stirring well three or four times. Filter into a graduated flask and wash with successive portions of water nearly boiling.

The reason for including this procedure is evident—to render the water-soluble potash insoluble before the extraction of the potash. The removal of phosphoric acid from solution by precipitation tends to carry down potash also, which Bible mentions as the probable cause of low results with the alternative official method[3]. The plan, therefore, commends itself as a practical way of avoiding this difficulty.

A careful test of this operation, during which the directions were followed strictly, showed that water-soluble phosphoric acid is not completely removed from solution, but may be found in the filtrate after the washing is completed. However, by the use of heat and a longer digestion it was found that the soluble phosphoric acid can be completely removed by this process. The directions for carrying out these procedures would mean only slight changes in the paragraph "Preparation of Solution" in *Methods of Analysis*. They are as follows:

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[1] *This Journal*, 1925, 8: 419, 420.
[2] *Ibid.*, 1926, 9: 192.
[3] *Methods of Analysis*, *A. O. A. C.*, 1925, 14.

FERTILIZER BRAND	POTASH GUARANTEED	POTASH FOUND	
		Regular Official Method	Official Method Modified with $CaCO_3$
	per cent	*per cent*	*per cent*
3-8-6	6.00	5.37 5.96 5.77 5.61	5.81 5.70
2-8-2	2.00	1.87 2.04 2.08 1.96	1.97 1.98
2-8-5	5.00	4.62 4.64 4.50 4.74	4.80 4.75
2-12-2	2.00	1.86 1.67 1.70 1.17	1.88 1.82
3-12-4	4.00	3.34 3.67 3.49 3.59	3.69 3.77
3-8-4	4.00	3.80 3.90 4.15 4.27	3.84 3.89
4-16-4	4.00	3.96 4.12 3.80 4.23	4.01 4.07
2-16-2	2.00	1.18 1.53 1.53 1.65 1.49	2.13 2.15
2-12-2	2.00	2.14 1.63 1.95 1.64 1.98 1.80	2.05 2.04
Laboratory mixture—acid phosphate and potash salts.	5.63	5.01 3.70 3.32 4.60	5.52 5.52 5.40

PREPARATION OF SOLUTION.

(a) *Mixed fertilizers.*—Place 2.5 grams of the sample in a beaker, add 2 grams of calcium carbonate and 75 cc. of water, heat carefully to the boiling point, and keep hot for a minute or two, rotating the beaker. Place on a water bath and evaporate to 25 cc., occasionally rotating the contents of the beaker. With a wash bottle of hot water, transfer the contents of the beaker to a 12.5 cm. filter, receiving the filtrate in a 250 cc. graduated flask. Wash with successive small portions of hot water until the filtrate amounts to 200 cc. Add to the hot solution 1 or 2 cc. of strong ammonium hydroxide and sufficient saturated ammonium oxalate solution to precipitate all the lime present, cool, dilute to 250 cc., mix, and pass through a dry filter.

(From this point, the directions for the method remain unchanged.)

Data on the use of this method with some commercial mixed fertilizers are given in the table. They were obtained by the regular official method and this modified method, in which calcium carbonate is used to render the phosphoric acid insoluble.

One great difficulty experienced by the writer with the official method, in which no steps are taken to remove phosphates, was a failure to get conclusive results. They were generally low, and when a number of determinations was run wide divergence was shown even in duplicates. On the other hand, some of the results may compare closely with those obtained by other methods, which would argue for their correctness. Thus, while the official method may give the true result, it is uncertain because agreeing duplicates may not have been obtained.

It is believed that some steps should be taken to modify the official method in order that the interfering action of phosphoric acid and water-soluble phosphates may be overcome. The writer would suggest that the Lindo-Gladding or regular official method be studied in comparison with a method specifying magnesium chloride and with the method outlined in this paper specifying calcium carbonate to remove the soluble phosphoric acid from solution. Such comparative study should be made upon known mixtures of acid phosphate and potash salts, and also on standard mixtures of materials occurring in complete fertilizers.

VARIATIONS IN FERTILIZER SAMPLES DRAWN BY OFFICIAL METHODS.

By L. D. Haigh (Agricultural Experiment Station, Columbia, Mo.).

Fertilizer chemists have been perplexed by the variations from the guarantee noted in some of the samples drawn. Whether these variations are due to failure of the official sampling method to obtain a true average sample, to faulty mixing, or perhaps to some segregating change in the contents of the sack after leaving the factory, is difficult to say definitely.

In 1913, the A. O. A. C. adopted directions for drawing samples of fertilizer that should be fully adequate to obtain a good average sample from any lot or stock. In brief, it specifies that the official samples shall be drawn, in most cases from 10 per cent of the sacks, by any sampling device that will obtain a core through the sack from top to bottom. The mixture of these drawn cores quartered down to one pound or more shall constitute an official sample. The sampler used has been described[1]. It has been found very effective for this work.

The official samples of the same mixtures from the same fertilizer factory often vary considerably. Some samples may show overrun of some one or more constituents, while other samples will show certain deficiencies in these same constituents. Generally speaking, if enough samples of any one kind have been obtained, they will average fairly close to the guaranteed value.

For example, seventeen official samples of a 1.65–12–2 fertilizer from a certain factory analyzed as follows:

SAMPLE	NITROGEN	AVAILABLE P_2O_5	POTASH
	per cent	*per cent*	*per cent*
1	1.68	12.49	2.06
2	1.71	12.13	1.93
3	1.64	12.47	2.11
4	1.74	12.51	2.03
5	1.68	11.72	2.22
6	1.63	12.96	1.79
7	1.66	12.33	1.58
8	1.69	12.57	1.43
9	1.70	12.09	1.40
10	1.74	12.03	2.02
11	1.55	12.07	1.75
12	1.49	11.92	2.29
13	1.66	12.12	1.58
14	1.64	12.02	1.46
15	1.59	12.13	2.56
16	1.68	12.08	1.82
17	1.69	11.60	2.01
Average	1.66	12.21	1.88

Thus it is found that compared with the guarantee six samples were deficient in nitrogen and eleven samples showed more or less overrun. In available phosphoric acid three samples run under and fourteen samples run over the guarantee in varying amounts. The large number of deficiencies in potash may be due to the fact that the present official method is apparently giving low results. The range of variations is wider with potash, while only one result shows a large overrun. Assuming that all these potash results should be somewhat higher, the average might be in the neighborhood of 2 per cent or better.

Still another set of samples of 1.65–12–2 gave the following results:

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[1] *This Journal*, 1923, **6**: 410.

SAMPLE	NITROGEN *per cent*	AVAILABLE P_2O_5 *per cent*	POTASH *per cent*
1	1.61	11.71	2.06
2	1.55	12.33	2.00
3	1.64	12.17	1.89
4	1.67	12.07	1.90
5	1.54	11.65	2.03
6	1.64	11.44	2.03
7	1.68	12,16	2.00
8	1.65	11.02	2.03
9	1.72	11.94	2.01
10	1.70	12.49	2.06
11	1.68	11.59	1.95
12	1.70	12.03	1.75
13	1.49	12.23	1.94
Average	1.64	11.91	1.97

The following results were observed on a set of samples of Acid Phosphate, 16 per cent, the first nine samples being over the guarantee and the others under:

	AVAILABLE PHOSPHORIC ACID *per cent*
1	18.44
2	16.33
3	16.04
4	16.30
5	17.58
6	16.72
7	18.27
8	16.07
9	17.06
10	14.96
11	15.97
12	15.97
13	15.49
14	15.66
15	15.99
16	15.60
17	15.61

The average of these 17 samples gives a value of 16.36 per cent available phosphoric acid, which would appear to be a fair and correct percentage for a season's output of this material.

As a preliminary inquiry into the cause of this variation, an attempt was made to study the composition of the individual sacks from which the official sample was taken. The following analysis of a lot of 2.47–8–6 fertilizer serves to illustrate the plan. The 12 sacks from which these samples were drawn proved to be quite uniform in composition.

The samples for the experiment were obtained as follows: The sampling tube was inserted four times into each sack of the lot making up the official sample, four cores being taken from the top to the bottom of the sack. Three of these were put together to make a sample representing the individual sack, and the other core was put with the single core sample from other sacks to make up the official sample. The results of the analyses of the individual sack samples, and the calculated average of these results are shown in comparison with the results on the corresponding official sample.

2.47–8–6 FERTILIZER.

		INSOLUBLE	AVAILABLE	
SACK	NITROGEN	P_2O_5	P_2O_5	POTASH
	per cent	*per cent*	*per cent*	*per cent*
1	2.49	1.24	8.49	6.20
2	2.48	1.29	8.45	6.18
3	2.33	1.21	8.82	6.15
4	2.51	1.30	8.78	6.35
5	2.45	1.46	8.22	6.34
6	2.31	1.75	8.20	6.34
7	2.29	1.31	8.74	5.82
8	2.45	1.13	8.61	6.34
9	2.46	1.20	8.46	6.30
10	2.49	1.25	8.36	6.52
11	2.43	1.29	7.72	6.04
12	2.36	1.28	8.69	5.68
Average	2.42	1.31	8.46	6.19
Guaranteed	2.47	0.50	8.00	6.00

The variations in composition between the different sacks are sometimes greater with some lots than with others. In most cases the analyses of the official sample, which is a composite of portions from all the sacks, runs quite close to the result obtained by averaging the analyses of each separate sack.

The 1.65–12–6 fertilizer illustrates a lot, the separate sacks of which varied considerably in composition. However, the average result and the result from the official sample are much the same.

Two lots of Half & Half Fertilizer (bone and acid phosphate mixture) were studied by analyzing the separate sacks. The first lot appeared to be a mixture in which the proportion of bone in all the sacks was too high, resulting in an excess of nitrogen and a deficiency of available phosphoric acid. The fine acid phosphate may have sifted out of the sack during transportation. In the second lot the mixture seemed to be more true to name, as the available phosphoric acid appeared to conform to the guarantee, and the excess of nitrogen was not quite so great.

Just what causes one sack to vary widely from another, though made of the same mixture and drawn from the same bin, is not easy to say with certainty. It seems logical that the jolting to which the sacks are subjected in transportation may have something to do with the variation in the individual sacks. Segregation probably takes place, the heavier and coarser particles rising to the top and the finer parts of the mixture settling to the bottom. Unless the sack is paper lined, some of the fine material may also be lost by working out through the meshes of the sack.

It will be readily seen that when the official sample from any lot is made up of sacks, the majority of which show individual underruns in any constituent, the official sample will also show an underrun of the same constituent. Official samples of other lots of the same brand may be made up of a majority of sacks which overrun in the same constituent. This will cause a correspondingly high result in the official sample when analyzed.

The following results of analyses of different mixtures were obtained by the writer:

Results of analyses of different fertilizers.

SACK	3.30–4–1 FERTILIZER				0.82–9–1 FERTILIZER			
	NITROGEN	INSOLUBLE P_2O_5	AVAILABLE P_2O_5	POTASH	NITROGEN	INSOLUBLE P_2O_5	AVAILABLE P_2O_5	POTASH
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	3.10	0.53	5.29	1.69	1.10	0.54	10.46	1.73
2	2.63	0.48	5.75	2.22	1.17	0.59	10.14	1.68
3	3.10	0.40	4.90	1.30	1.15	0.63	10.07	2.25
4	2.57	0.44	5.80	2.58	1.01	0.71	10.97	1.88
5	3.27	0.42	4.46	1.63	1.02	0.73	10.61	2.03
6	2.70	0.75	6.21	2.82	1.09	0.67	10.25	1.75
7	2.61	0.75	6.19	2.43	1.16	0.71	9.29	1.80
8	2.92	0.39	4.95	0.89	1.03	0.78	10.09	1.96
9	3.12	0.54	5.26	1.18	1.03	0.90	9.83	1.69
10	3.21	0.59	5.64	1.53	1.07	0.93	9.68	1.75
Average	2.92	0.53	5.44	1.83	1.08	0.72	10.14	1.85
Official sample	2.92	0.64	4.85	1.66	1.10	0.45	10.53	1.52
Guaranteed	3.30	0.10	4.00	1.00	0.82	. . .	9.00	1.00
	1.65–10–2 FERTILIZER				ACID PHOSPHATE FERTILIZER			
1	1.61	1.68	10.04	2.07	....	1.81	17.10	. .
2	1.61	1.63	9.88	2.01	....	2.06	16.18	.
3	1.62	1.66	9.87	1.99	....	1.80	17.11	
4	1.59	1.64	9.89	2.06	....	1.61	16.68	. .
5	1.69	1.57	10.24	2.08	....	1.65	16.66	.
6	1.85	1.57	9.64	2.85	. .	1.90	16.37	. .
7	1.78	1.48	10.69	2.02	....	1.77	16.61	....
8	1.60	1.61	9.83	2.03	....	1.84	16.14	....
9	1.71	1.52	10.37	1.98	...	1.86	16.11	....
10	1.77	1.66	10.57	2.00	....	1.81	16.66	.
11	....	....	....	. ..	...	1.70	15.41	....
Average	1.68	1.60	10.10	2.11	....	1.80	16.46	....
Official sample	1.69	1.32	10.43	2.02	....	1.86	16.33	.. .
Guaranteed	1.65	1.00	10.00	2.00	....	0.50	16.00	. .
	1.65–12–6 FERTILIZER				1.65–8–2 FERTILIZER			
1	0.91	1.19	13.51	5.20	1.51	2.40	7.76	2.30
2	0.42	0.58	16.94	3.23	1.52	2.31	8.08	2.09
3	2.49	1.19	12.11	4.78	1.52	2.25	7.50	2.22
4	2.55	1.46	10.88	7.90	1.55	2.35	7.89	2.24
5	0.95	1.09	12.26	8.34	1.59	2.25	7.90	2.27
6	0.56	0.44	13.88	11.66	1.66	2.30	8.18	2.35
7	0.51	0.32	13.84	12.45	1.55	2.25	7.94	2.11
8	0.72	0.48	14.13	10.31	Lost	. . .	....	....
9	1.05	0.85	14.06	3.50	1.54	2.14	7.88	2.12
10	1.06	1.24	11.96	2.98	1.53	2.13	7.81	2.11
Average	1.12	0.88	13.36	7.04	1.55	2.27	7.88	2.20
Official sample	0.98	1.01	13.37	7.04	1.54	2.03	8.18	2.07
Guaranteed	1.65	0.50	12.00	6.00	1.65	0.50	8.00	2.00

Results of analyses of different fertilizers—Continued.

SACK	1.65-12-2 FERTILIZER SAMPLE No. 1				HALF AND HALF FERTILIZER 1 23-13			
	NITROGEN	INSOLUBLE P_2O_5	AVAILABLE P_2O_5	POTASH	NITROGEN	INSOLUBLE P_2O_5	AVAILABLE P_2O_5	POTASH
1	1.73	2.30	12.40	2.63	1.87	11 94	10.60	....
2	1.68	2.36	12.02	2.45	2.07	11.61	11.03	...
3	1.71	2.39	12.61	2.54	1.80	12.43	10.41	
4	1.59	2.31	11.98	2.44	1.90	11.59	9.34	...
5	1.67	2.28	11.84	2.63	1.82	12.22	9.03	....
6	1.68	2.29	11.99	2.65	1.97	13.38	8.87	. .
7	1.72	2.49	10.77	2.55	1.86	11.32	9.76	...
8	1.85	2.51	11.26	2.82	1.96	11.77	9.08	...
9	1.66	2.30	12.15	2.51	1.98	13.02	8.62	.
10	..	...	. .	. .	2.38	13.02	8.81	...
Average	1.69	2.36	11.89	2.58	1.96	12.23	9.56	. ..
Official sample	1.65	2.38	12.27	2.53	1.95	12.12	9.62	....
Guaranteed	1.65	1.50	12.00	2.00	1.23	7.00	13.00	....
	1.65-12-2 FERTILIZER SAMPLE No. 2				HALF AND HALF FERTILIZER 1.23-12			
1	1.72	1.62	12.98	1.16	1.18	6.94	14.78	
2	1.60	1.48	13.12	1.50	1.61	7.89	12.14	. .
3	1.68	1.50	13.20	1.53	1.64	7.53	12.57	
4	1.69	1.46	13.31	1.51	1.81	8.80	11.44	
5	1.70	1.39	13.52	1.52	1.65	7.94	12.20	
6	1.67	1.62	12.95	1.33	1.74	8.77	12.00	. .
7	1.65	1.53	13.02	1.35	1.91	9.48	11.16	
8	1.66	1.55	13.14	1.15	1.68	8.12	12.37	
9	1.69	1.39	13.21	1.91	1.80	8.53	12.07	.
10	1.72	1.51	13.13	1.47	1.67	7.52	12.72	
Average	1.68	1.51	13.16	1.44	1.67	8.15	12.35	.
Official sample	1.67	1.51	13.80	2.09*	1.64	8.62	12.02	. .
Guaranteed	1.65	0.50	12.00	2.00	1.23	8.00	12.00	...

* Result not verified.

A 1.65–12–4 fertilizer was also analyzed. The following results, expressed in percentage, were obtained as an average for 12 sacks:

	NITROGEN	INSOLUBLE P_2O_5	AVAILABLE P_2O_5	POTASH
Average........	1.72	1.22	11.60	4.95
Official sample....	1.70	1.05	12.12	4.85
Guaranteed	1.65	....	12.00	4.00

REPORT ON PLANTS.

By A. J. PATTEN (Agricultural Experiment Station, East Lansing, Mich.), *Referee.*

The role of minerals in animal nutrition is now known to be of far greater importance than was generally believed a decade or so ago. In addition to being the principal material from which the frame work of the body is constructed, recent investigations indicate that the presence of certain minerals, even in very minute quantities, may be essential or detrimental to the proper functioning of many of the vital processes of life.

J. S. McHargue[1] of the Kentucky Agricultural Experiment Station has published a number of papers showing that small quantities of copper, manganese, zinc, nickel, and cobalt are widely distributed in soils and plants, and has pointed out their possible functions as vital factors. Other investigators, from time to time, have reported finding arsenic, antimony, cadmium, strontium, bromine, iodine, and fluorine in small quantities in plants distributed over wide areas.

All of which makes the subject of plant analysis of far greater importance than ever before, and also presents an entirely different aspect to the subject. Since all these elements are present in plants in very small quantities, the necessity for methods that will permit of accurate determinations under such conditions is readily apparent.

During the past year the referee has not had time to give attention to any phases of the problem except the methods for iron and aluminum. No cooperative work has been requested, because it was felt that more research must be done before resubmitting them for cooperative study.

A number of methods for the direct determination of aluminum have appeared in the literature in recent years. Such a method, if reliable, would be welcomed and would be a distinct advantage over the present method.

The Schmidt-Hoagland method[1] has been rather extensively used in biological work. In this method the aluminum is precipitated as the phosphate after the iron is reduced to the ferrous state by the addition of ammonium thiosulfate. Without at this time presenting any evidence, it may be simply stated that the referee has not obtained satisfactory results with this method on plant material. The final precipitate always contains traces of iron and calcium, and there has been no certainty that all the aluminum was precipitated.

The colorimetric method recommended by Atack[2], specifying sodium alizarine sulfonate (alizarine S) has also been studied. The formation of a colored lake of aluminum with the dye is the basis of this method.

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[1] *J. Agr. Res.*, 1925, **30**: 193; *J. Am. Soc. Agr.*, 1925, 17. 368.
[2] *J. Soc. Chem. Ind.*, 1915, **34**: 936.

It was found that other metals, notably iron, greatly interfered with the color reaction, and no satisfactory results could be obtained.

More recently, three Dutch investigators[1] recommended the use of sodium alizarate, which they claimed to be superior to sodium alizarine sulfonate. However, they state that the color reaction is disturbed by both ferrous and ferric iron and that magnesium gives about the same color reaction as aluminum.

F. S. Williamson[2] studied the reaction between sodium alizarate and aluminum and came to the conclusion that no definite compound is formed, but rather an adsorption complex. It seemed useless, therefore, to investigate the method further.

Lundell and Knowles[3] have published two articles on the determination of small quantities of aluminum in nonferrous metals. One of the methods proposed by them is a colorimetric determination with the dye aurin tricarboxylic acid recently described by Hammett and Sottery[4]. This method has not been investigated, but its application to the determination of aluminum in plants should receive attention.

REVISION OF METHODS.

There is urgent need for revising some of the methods that are now official. In his address to the association last year, C. A. Browne pointed out very clearly the need for revising the methods for chlorine. This should be done as soon as a referee can be found to undertake the work.

The methods of preparing samples of plant material for analysis should also be carefully investigated. The present official method directs that all foreign matter, especially adhering soil, be removed thoroughly without stating how this shall be done. It has come to the attention of the referee that the practice of washing the plant material with water to remove adhering sand is used by some investigators.

It would seem that this practice might introduce an error that would be greater than that due to a small quantity of unremoved foreign material.

All these problems need to be investigated.

RECOMMENDATIONS[5].

It is recommended—

(1) That the methods for the determination of iron and aluminum in plants be further studied.

(2) That a referee be appointed to study methods for the determination of copper, zinc, nickel, cobalt, and other so-called "rare elements" in plants.

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[1] *Chem. Weekblad*, 1923, **20**: 193.
[2] *J. Phys. Chem.*, 1924, **28**: 891.
[3] *J. Ind. Eng. Chem.*, 1925, **17**: 78; 1926, **18**: 60
[4] *J. Am. Chem. Soc.*, 1925, **47**: 142
[5] For report of Sub-committee A and action of the association, see *This Journal*, 1926, **10**. 65.

(3) That a referee be appointed to study methods for the determination of total chlorine in plants.

(4) That a referee be appointed to study methods for the preparation of samples for analysis.

A MODIFICATION OF THE SALICYLIC-THIOSULFATE METHOD SUITABLE FOR THE DETERMINATION OF TOTAL NITROGEN IN PLANTS, PLANT SOLUTIONS, AND SOIL EXTRACTS.

By EMERY R. RANKER (Bureau of Plant Industry, Department of Agriculture, Washington, D. C.).

The determination of total nitrogen in plants and plant solutions presents problems that challenge the accuracy of the various methods used. For example, whole green plants including the residual nutrient solution in which they grew comprise a typical sample, and one in which practically every kind of nitrogen is present. In the plants amino, amide, and some ammonia nitrogen are present in their various combinations and proportions. In the residual nutrient solution nitrate, and possibly ammonia nitrogen, is present in addition to other forms of nitrogen produced by bacterial action upon the sloughed-off root cap cells and other organic matter present.

On the plant side, the need for a reliable method for the determination of total nitrogen is evidenced by a consideration of the literature on the problem of nitrogen fixation by plants. In this field there is much controversy. The significance of the results presented, both pro and con, may be judged to a large extent by the accuracy of the particular method used in the estimation of nitrogen. This condition indicates the need of more reliable methods of analysis for plant materials.

At present two methods are in general use: (1) the Gunning method modified to include the nitrogen of nitrates, which in this paper will be called the salicylic-thiosulfate method; and (2) some method involving the use of Devarda alloy.

In the presence of organic matter all modifications of the Devarda method for the estimation of total nitrogen are time-consuming, because there is a preliminary alkaline distillation followed by a Kjeldahl digestion and a subsequent second distillation into the same or a second lot of standard acid. On the other hand the salicylic-thiosulfate method is criticized severely by several investigators whose data have been interpreted as indicating the limitations and defects of any method based on the reduction of nitrates in acid medium[1]. Unfortunately, then, neither of these methods is entirely satisfactory for the determination of total nitrogen in plants and plant solutions.

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[1] These criticisms have been analyzed and considered previously (*Ann. Missouri Botan. Garden*, 1925, 12: 373. A critical study of the data upon which these criticisms are based reveals the fact that the moisture content of the samples was not adequately controlled.

During certain studies in plant nutrition the salicylic-thiosulfate method, as given for the determination of total nitrogen in fertilizers[1], was used to estimate the total nitrogen present at the end of the experiments. When applied to materials of the kind under consideration, extremely inaccurate results were obtained. From nutrient solutions containing from 10–400 mg. of nitrogen per 950 cc., only 27–68 per cent of the nitrogen was recovered, as shown by the data of Table 1.

TABLE 1.

Recovery of nitrogen by the salicylic-thiosulfate method.

NITROGEN PRESENT PER 950 CC.	RECOVERY
mg.	*per cent*
10	27
25	54
50	62
100	62
200	30
300	67
400	68

From solutions containing quantities of nitrogen smaller than 10 mg. the amounts recovered were usually less than for the blank, that is, there was a loss of nitrogen from the reagents used. A total of about 60 determinations was made, but no agreement among the results was obtained. The greatest losses of nitrogen occurred in those samples that contained whole plants in addition to the residual nutrient solutions. In several cases a visible evolution of nitrogen dioxide fumes occurred, and duplicate determinations varied widely. These data tend to corroborate the statements of those investigators who have criticized such methods as the salicylic-thiosulfate, the accuracy of which depends upon the reduction of nitrates in an acid medium.

Some preliminary tests, however, seemed to indicate that the inaccurate results obtained by these methods when used for plant work were due, primarily, to the presence of free water at some stage during the process of acid digestion. Also, certain details of manipulation seemed to influence the determination in certain cases. Based upon these preliminary tests a modification was devised, and its accuracy for the determination of total nitrogen was tested out on the various forms of nitrogen. For purposes of comparison, simultaneous determinations on samples from the same stock, measured by the same pipets and at the same temperature, were made by a modification of the Devarda method, hereafter referred to as the comparison method. The procedure for this comparison method, a description of the materials used, and other details of the experiment including the results obtained have been published. Reference has been given previously in this paper. Certain of these results are of interest here and are given in Table 2.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 9.

TABLE 2.

Recovery of total nitrogen.

(Average percentages and probable error.)

SAMPLE DETERMINED	RECOVERY BY COMPARISON METHOD (A MODIFICATION OF THE DEVARDA METHOD)	RECOVERY BY A MODIFICATION OF THE SALICYLIC-THIOSULFATE METHOD		NO. OF TRIALS
		Sample first evaporated to dryness	Sample plus water	
50 mg. nitrate nitrogen	97.7 ±0.19	100.4 ±0.06	62.2 +0.38	8
100 mg. nitrate nitrogen	68.5* 99.9‡ ±0.24	99.2 ±0.10	17.8 ±0.81	13 3
50 mg. nitrate nitrogen 10 mg. ammonia nitrogen	98.5 ±0.23	98.0 ±0.06	36.7 ±1.80	12
50 mg. nitrate nitrogen 10 mg. amino nitrogen	97.7 ±0.14	99.3 ±0.14	51.7 ±4.03	12
50 mg. nitrate nitrogen 10 mg.–amino nitrogen –amide nitrogen	97.5 ±0.09	99.3 ±0.28	47.6 ±0.29	15
50 mg. nitrate nitrogen 7 mg. plant nitrogen†	99.6 ±0.09	100.2 ±0.32	64.6 ±0.23	12
50 mg. nitrate nitrogen 7 mg. plant nitrogen† 0.5 cc. H_2SO_4	These samples were not determined, as during evaporation heavy NO_2 fumes were given off.			
50 mg. nitrate nitrogen 7 mg. plant nitrogen†‖	100.3 ±0.56	99.7 ±0.69	..	14
50 mg. nitrate nitrogen 7 mg. plant nitrogen† 1 cc. 0.1 *N* NaOH	99.2 ±0.52	99.1 ±0.62		6
50 mg. nitrate nitrogen 0.5 gram sucrose	98.1 ±0.64	92.7 ±0.90	33.2	10

* 1.0 gram of Devarda alloy used.
† Supplied as the nitrogen content of ten 6-day-old wheat seedlings.
‡ 2.0 grams of Devarda alloy used.
‖ This solution was adjusted to neutrality prior to determination.

From a study of the data of Table 2 certain facts are evident:

1. The presence of free water in the sample is the determining factor for the accuracy of a method dependent upon the reduction of nitrates in acid medium.

2. When the sample is practically dry the salicylic-thiosulfate method as here modified is somewhat more accurate for the determination of total nitrogen than the comparison method used.

3. The modified method is accurate for the determination of amino, amide, ammonia, nitrate, and total plant nitrogen, and combinations of these forms in plants and plant solutions.

4. If sugar is present in abundance, a slight loss of nitrate nitrogen may occur, owing to the reducing action of the sugar. This loss would be very slight in actual practice since the nitrate-nitrogen content of plants is small, as shown by the data of Table 3.

TABLE 3.

Determination of total nitrogen in samples of high sugar content.

(Average percentages and probable error)

NO.	SAMPLE USED	ANALYSIS BY COMPARISON METHOD	ANALYSIS BY SALICYLIC-THIOSULFATE MODIFIED METHOD	NO. OF TRIALS
		mg.	*mg*	
19	Sugar beet (water extract)	10.9 ± 0.067	10.7 ± 0.029	15
18	Sugar cane (water extract)	11.4 ± 0.045	11.5 ± 0.006	15

The modification of the salicylic-thiosulfate method that gives the most accurate results on plants and plant materials, and the one recommended, is as follows:

Place the sample in an 800 cc. Kjeldahl flask and adjust to neutrality or make slightly alkaline; if water is present, evaporate *just to* dryness on a water bath under vacuum. Add 35–40 cc. of salicylic acid mixture (1.0 gram of salicylic acid to 30 cc. of concentrated nitrogen-free sulfuric acid), mix thoroughly, and allow to stand for at least an hour with occasional shaking. (If organic matter is present, stopper tightly with a rubber stopper and allow to stand overnight.) Add 5 grams of sodium thiosulfate and heat for 5 minutes with a low flame, and cool. Add 7–10 grams of anhydrous sodium sulfate and a pinch of copper sulfate. Digest for an hour at the boiling point after the solution clears, and just before the solution solidifies dilute to an estimated volume of 400 cc., and cool completely. Add a small piece of paraffin, 100 cc. of a saturated solution of sodium hydroxide, and a piece of mossy zinc; connect immediately with the distillation apparatus and distil 150–200 cc. over into standard acid during a period of 1 hour. Titrate the standard acid to neutrality with standard alkali, using methyl red indicator, and calculate the amount of nitrogen present.

This method will be referred to in this paper as "the modified method". The remaining pages will be devoted to the presentation and consideration of data (1) which demonstrate the accuracy and applicability of this method, (2) which illustrate the influence of certain details of manipulation upon the accuracy of such methods, and (3) which indicate the desirability of suitable qualitative tests, for the loss of nitrogen, as control measures.

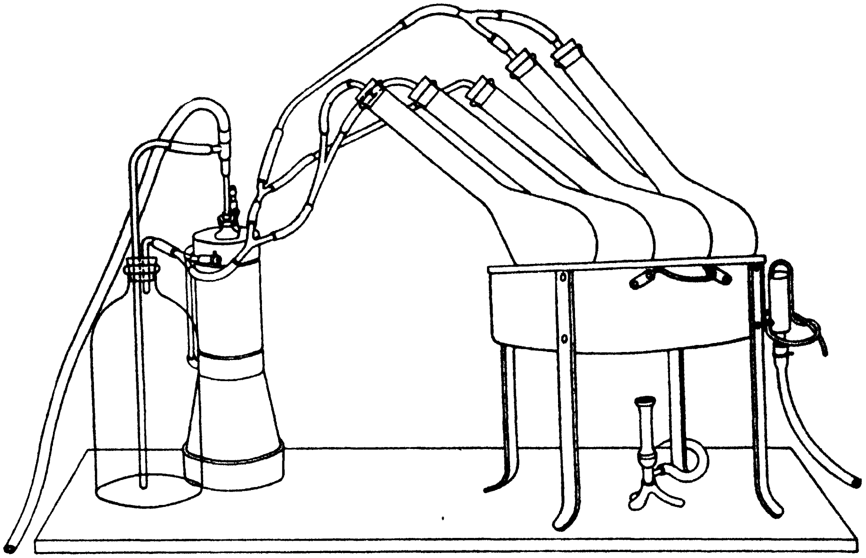


FIG. 1.—APPARATUS USED FOR THE EVAPORATION OF SAMPLES UNDER VACUUM.

MATERIALS.

Most of the materials used in this investigation were in the form of extracts when analyzed. At the time of sampling, the solutions were free from any precipitate or suspended matter. All samples were measured into 800 cc. Kjeldahl flasks with the same pipet, at the same time and temperature, and all other conditions of making the samples were as nearly identical as possible. The numbers assigned to the various materials correspond to similar numbers used in the tables. Though two sets of samples, as given in the various tables, may bear the same number they are not comparable, necessarily, unless they appear in the same table; they may have been measured out at different times and temperatures. The stock materials from which samples were taken are as follows:

1—*Aspergillus niger* (whole cultures including residual solutions)	These organisms were cultured in 100 cc. flasks containing exactly 25 cc. Pfeffer's nutrient solution to which 1 per cent glucose had been added. The cultures were grown until a vigorous heavy mat had formed. The entire contents of 1 culture flask constituted 1 sample, being transferred without loss to a Kjeldahl flask for determination of nitrogen.
2—*Fusarium culmorum* (whole cultures including residual solutions)	

3—*Aspergillus niger* (residual solution only)	These organisms were cultured in 300 cc. flasks containing 100 cc. of nutrient solution (refer to Nos. 1 and 2). At time of sampling the cultures were boiled for 5 minutes and filtered. Analyses were made of 25 cc. of the clear filtrate per sample.
4—*Fusarium culmorum* (residual solution only)	
5—*Phoma Betae* (residual solution only)	
6—Tobacco leaves (water extract)	The fresh material was ground through a food chopper, any drippings produced being added; 1 volume of water, or 1 volume of 70 per cent alcohol, was added, the mixture was boiled (refluxed in the case of alcohol) for 20–30 minutes and filtered hot; 25 cc. samples were taken from the filtrate for analyses.
7—Geranium leaves (water extract)	
8—Pea leaves and terminals (water extract)	
9—Celery leaves and stalks (water extract)	
10—Algae mixture, mostly Spirogyra (water extract)	
11—Tomato fruits, ripe (water extract)	
12—Tobacco leaves and tops (alcohol extract)	
13—Geranium leaves (alcohol extract)	
14—Pea leaves and terminals (alcohol extract)	
15—Celery leaves and stalks (alcohol extract)	
16—Tomato fruits, ripe (alcohol extract)	
17—Greenhouse soil plus mushroom compost (water extract)	1 kg. dry material plus 1 liter water stirred rapidly 4 hours, allowed to precipitate, decanted, filtered. 25 cc. samples taken from filtrate.
18—Sugar cane (water extract)	These materials were prepared in the same manner as indicated for Nos. 6–16.
19—Sugar beet (water extract)	
20—Pea leaves and terminals (water extract)	
21—Greenhouse soil containing mushroom compost (water extract)	Prepared from same materials and in same manner as No. 17.

22—Mushroom compost (only) (water extract)	Prepared same as No. 17.
23—Mushroom compost (autoclave extraction)	Prepared same as No. 17 except the mixture was autoclaved, not stirred.
24—Mushroom compost, KNO_3	25 cc. No. 22 and 74 each, per sample.
25—*Aspergillus niger*, KNO_3	25 cc. No. 3 and 74 each, per sample.
26—*Fusarium culmorum*, KNO_3	25 cc. No. 4 and 74 each, per sample.
27—Crude peat (water extract)	Prepared same as Nos. 6–16.
28—Sphagnum moss (dry) (water extract)	
29—*Selaginella apus* (water extract)	
30—*Selaginella apus* (expressed sap)	
32—*Selaginella apus* (water extract)	
33—	These numbers refer to various samples of solution cultures of barley, wheat, and peas. These materials were used for qualitative analysis only; the nature and preparation of the samples are indicated in the various tables where the data of their analyses are recorded.
34—	
35—	
36—	
37—	
38—	
39—	
40—	
41—	
42—	
43—	
44—	
45—	
46—	
47—	
48—	
49—	
50—	
52—Alanine, KNO_3, solution	25 cc. alanine solution (1.277 gram per liter) and No. 74 each, per sample.
53—Asparagine, KNO_3, solution	25 cc. asparagine solution (0.873 gram per liter) and No. 74 each, per sample.
54—Sugar cane, KNO_3	25 cc. No. 18 and 74 each, per sample.
60—Sugar beet (water extract)	Prepared same as Nos. 6–16.
73—Heavy clay-loam soil (water extract)	Prepared same as No. 17.

74—KNO_3 solution	This solution contained 1.443 grams of potassium nitrate per liter; by analysis, 25 cc. contained 4.79 ±0.005 mg. of nitrogen. In all cases those samples that contained *added* nitrate nitrogen received 25 cc. of this solution. The nitrogen content of this solution did not vary throughout the period of these investigations, as determined by frequent control analyses of 25 cc. samples.

METHODS.

Total nitrogen was determined by two methods: (1) the modified method, and (2) the modification of the Devarda method to which reference has been made, and which was used as a comparison method.

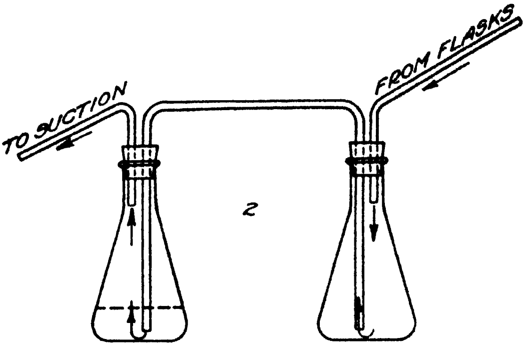
In addition to the duplicate analyses by the comparison method, the accuracy of the modified method was checked by three suitable qualitative tests for the loss of nitrogen as follows:

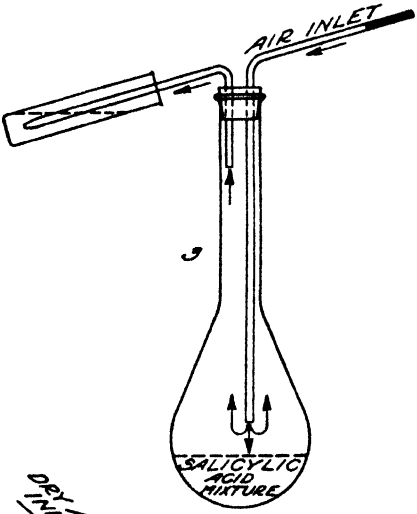
1. *Qualitative tests made during the process of evaporation of the sample under vacuum.* All the vapors evolved in this process were passed through a weak solution of sodium bicarbonate and collected in a second flask, both of which are illustrated in Fig. 2. The solution thus collected was tested, (A) for the presence of nitrites and nitrates by the diphenylamine test, and (B) for the presence of ammonia by the use of Nessler's reagent.

2. *Qualitative tests made following the addition of the salicylic acid mixture to the sample.* Any vapors or fumes formed during this process were forced through a weak solution of sodium bicarbonate by gently blowing on the air inlet tube of the device illustrated in Fig. 3. The solution thus obtained was tested, (A) for the presence of nitrites and nitrates by the diphenylamine test, and (B) for the presence of ammonia by the use of Nessler's reagent.

3. *Qualitative tests made during the process of acid digestion.* The fumes evolved during this process were led through and collected in a flask containing approximately 50 cc. of distilled water. The solution thus collected was tested for the presence of nitrites, nitrates, and ammonia as previously indicated [No. 2 (A) (B)].

The acid and alkali used in titration were standardized against benzoic acid obtained from the U. S. Bureau of Standards (Sample No. 39B). Fiftieth normal acid and alkali were used in all titrations. The normality factors were redetermined frequently to avoid possible errors from this source. Methyl red was used as the indicator, and all titrations were carried to the complete disappearance of any red tinge. This end point is practically identical with that of cochineal, and when 0.02 *N* alkali is used methyl red is much more sensitive.





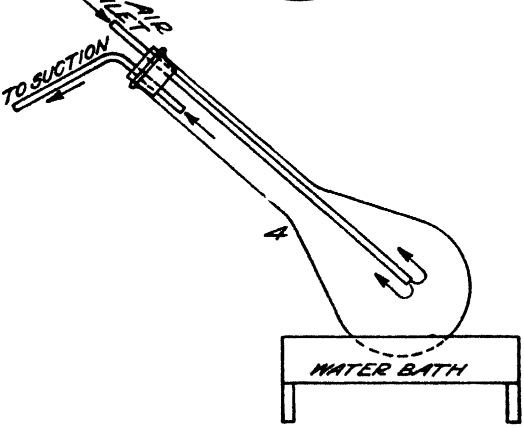


FIG. 2.—ARRANGEMENT OF FLASKS FOR COLLECTION OF SAMPLES TO TEST FOR LOSS OF NITROGEN DURING THE PROCESS OF EVAPORATION UNDER VACUUM.

FIG. 3.—ARRANGEMENT FOR COLLECTING SAMPLES TO TEST FOR LOSS OF NITROGEN FOLLOWING ADDITION OF THE ACID MIXTURE TO THE KJELDAHL FLASK.

FIG. 4.—DEVICE USED FOR THE EVAPORATION OF WHOLE GREEN PLANTS; THIS IS WELL ADAPTED FOR USE WITH PULPY MATERIALS ALSO.

All probable errors reported in this paper were calculated according to the following formula:

$$\left(E_m = \frac{0.6745\sigma}{\sqrt{n}}\right).$$

For evaporation of the samples a 5 hole, constant-level, gas-heated water bath was used; the flasks were held on this bath in an inclined position by two notched wooden supports and connected with an ordinary water filter-pump by a series of five stoppers (rubber) and four Y-tubes. A 3 liter safety bottle was placed between the filter-pump and the flasks on the water bath to prevent the entrance of water into the flasks when, for any reason, the water pressure was reduced. In operation, a partial vacuum was quickly developed, and ebullition proceeded at a rapid rate, provided all rubber connections were sufficiently thick-walled to withstand the vacuum developed. The vacuum should be released slowly before removing the flasks; if released rapidly, the flasks will crack. This apparatus is illustrated in Fig. 1.

EXPERIMENTAL.

Throughout this report the term "vacuum" has reference to an equivalent of not less than 22–23 inches of mercury. By the term "just to dryness" reference is made to that stage in the evaporation process at which the sample has *just ceased* ebullition and there may or may not be water of condensation in the neck of the flask. The term "ash dryness" applied to evaporation is used to indicate that condition in which the sample, the entire inside of the body of the flask, and most of the neck of the flask are dry. Evaporation to "partial dryness" denotes a condition in which some free moisture is present in the sample when evaporation is stopped.

In connection with the data of Table 4 it will be noted that the probable errors for determinations by the modified method are entirely satisfactory. The quantitative results are checked by qualitative tests so that the accuracy of the procedure is definitely known. There are a few cases of rather serious disagreement, however, between the results obtained by the modified method and the comparison Devarda method. The determinations for Sample No. 6 (tobacco leaves) are noticed at once. This discrepancy seems to exist, also, with the alcohol extract of the material, that is, the determination by the comparison method is low. It was determined that this discrepancy was due to the volatile nature of the nicotine contained in the tobacco material. During the alkaline distillation with Devarda's alloy a large part of the nicotine present is volatilized and driven over into the standard acid sample; some of it is absorbed but much of it passes out into the surrounding

TABLE 4.

*Accuracy of the modified method for total nitrogen in various samples of plant materials and soil extracts**.

(Arithmetical mean and probable error.)

SAMPLE USED		NUMBER OF TRIALS	ANALYSIS BY COMPARISON METHOD	ANALYSIS BY MODIFIED METHOD
NO.	DESCRIPTION			
			mg.	*mg.*
1	*Aspergillus niger* (whole culture including solution)	15	2.2 ± 0.014	2.3 ± 0.009
2	*Fusarium culmorum* (whole culture including solution)	15	2.3 ± 0.024	2.4 ± 0.01
3	*Aspergillus niger* (residual solution only)	17	0.84 ± 0.024	0.83 ± 0.005
4	*Fusarium culmorum* (residual solution only)	18	1.4 ± 0.021	1.4 ± 0.013
5	*Phoma Betae* (residual solution only)	15	2.1 ± 0.011	2.1 ± 0.03
6	Tobacco leaves (water extract)	15	11.4 ± 0.027	12.6 ± 0.039
7	Geranium leaves (water extract)	15	3.7 ± 0.028	3.9 ± 0.02
8	Pea leaves and terminals (water extract)	14	21.6 ± 0.045	21.8 ± 0.021
9	Celery leaves and stalks (water extract)	15	13.1 ± 0.03	13.6 ± 0.009
10	Algae mixture (water extract)	15	0.9 ± 0.003	0.9 ± 0.005
11	Tomato fruits (ripe) (water extract)	15	10.0 ± 0.018	10.0 ± 0.021
12	Tobacco leaves and tops (alcohol extract)	15	5.3 ± 0.028	5.5 ± 0.007
13	Geranium leaves (alcohol extract)	15	6.0 ± 0.039	6.2 ± 0.011
14	Pea leaves and terminals (alcohol extract)	15	9.0 ± 0.039	9.7 ± 0.015
15	Celery leaves and stalks (alcohol extract)	15	6.1 ± 0.027	6.0 ± 0.018
16	Tomato fruits (ripe) (alcohol extract)	15	15.2 ± 0.009	15.2 ± 0.012
17	Greenhouse soil plus mushroom compost (water extract)	15	3.9 ± 0.013	3.9 ± 0.006
20	Pea leaves and terminals (water extract)	15	29.3 ± 0.053	29.3 ± 0.014

* Qualitative tests by both diphenylamine and Nessler reagents showed no loss of nitrogen either during digestion in the comparison method or during evaporation and digestion in the modified method.

atmosphere. This condition is apparent from the odor of the nicotine given off, but it may be determined more or less quantitatively by collecting such vapors in sulfuric acid and subsequent analysis by the Kjeldahl method. Certain other disagreements exist, for example in Samples Nos. 6, 9, 12–14. In this connection attention must be directed to the rather outstanding fact that in all cases (except Sample No. 6 as noted above) where disagreement is evidenced, the relative magnitude of the probable error obtained by the modified official method indicates its greater accuracy and reliability. This fact is shown in the following instances: Sample No. 9 (13.1 ± 0.03 as compared with 13.6 ± 0.009); Sample No. 12 (5.3 ± 0.028 as compared with 5.5 ± 0.007); and Sample No. 13 (6.0 ± 0.039 as compared with 6.2 ± 0.011). An examination of other sets of determinations reveals the same evidence. The disagreement existing in the case of Sample No. 8 does not appear in the case of Sample No. 20, although both samples were taken from similar materials. Taken as a whole the data, which are based on a sufficiently large number of determinations, indicate rather satisfactory agreement between the methods, many of the determinations being identical, but the superior accuracy of the modified method is indicated.

To test further the accuracy of the method and its applicability to a larger number of plant materials and soil extracts, many determinations were made according to the procedure recommended, except that the processes of distillation and titration were omitted. This eliminated possible errors from these sources and allowed the distinctive processes of the method to be tested for loss of nitrogen under more exacting qualitative conditions. The tests were made, as indicated previously in the discussion of methods, to detect any loss of nitrogen during evaporation, on addition of acid, or during digestion of the sample. Each separate sample was thus tested five times. Negative tests were obtained for all samples, some of which are the following:

NO.	DESCRIPTION
21	Greenhouse soil
22	Mushroom compost (cold water extract)
23	Mushroom compost (autoclave extraction)
24	Mushroom compost extract plus KNO_3 solution
25	*Aspergillus niger* extract plus the residual solution plus KNO_3 solution
26	*Fusarium culmorum* extract plus the residual solution plus KNO_3 solution
27	Crude peat extract (boiled)
28	*Sphagnum* moss extract
30	*Selaginella expressed sap*
31	*Selaginella alcohol extract*
32	*Selaginella* extract
33	Barley plants (green) plus 10 cc. residual solution; high in nitrate nitrogen
34	Same as No. 33 except much lower in nitrate nitrogen
35	Wheat plants (green) plus 10 cc. residual solution; high in nitrate nitrogen
36	Same as No. 35 except much lower in nitrate nitrogen
37	Pea plants (green) plus 10 cc. residual solution; high in nitrate nitrogen

NO.	DESCRIPTION
38	Same as No. 37 except much lower in nitrate nitrogen
39	Residual solution from wheat cultures containing 200 mg. nitrate nitrogen
40	Same as No. 39 except containing 100 mg. nitrate nitrogen
41	Same as No. 39 except containing 50 mg. nitrate nitrogen
42	Same as No. 39 except containing 25 mg. nitrate nitrogen
43	Same as No. 39 except containing 10 mg. nitrate nitrogen
44	Same as No. 39 except containing 5 mg. nitrate nitrogen
45 46 47 48 49 50	These samples were the same as those of Nos. 39–44 except that barley plants were used instead of wheat.
52	Alanine plus KNO_3 solution
53	Asparagine plus KNO_3 solution.
54	Sugar cane extract plus KNO_3 solution
60	Sugar beet extract

These samples present a wide range of nitrogen content and combination. All contained some nitrate nitrogen, and many of them contained large amounts of nitrate nitrogen, which was reduced to ammonia in acid medium. The uniformly negative results obtained indicate that there was no loss of nitrogen during any part of the procedure. In other words, when the sample is properly neutralized and evaporated just to dryness under vacuum there is no loss of nitrogen. That the method will recover the nitrogen thus held is demonstrated by the quantitative data presented in Table 4.

Early in this investigation it became apparent that certain details of manipulation influenced the accuracy of the results obtained. In some cases the exact procedure to be followed was determined by these details. The qualitative tests that were developed for loss of nitrogen make it possible to determine the influence of certain details of procedure upon the accuracy of nitrogen methods. This phase of the subject was given some attention. Many of the samples were subjected to extreme conditions in order to determine the limits to which certain practices could be carried. The data from these various studies are presented in Tables 5–8 and immediately following Table 8.

It is evident from the data of Table 5 that the neutrality of the sample is a very important consideration with most of the samples (Nos. 5 and 5b, 29 and 32, 17 and 21, etc.). For such samples this one factor of neutrality would determine the accuracy of any quantitative analyses attempted. The samples just referred to were on the acid side of neutrality prior to neutralization; the alkaline reaction of Sample No. 26 produced a loss of ammonia in some of the five tests made (data of Sample No. 26a). Another effect of this factor of neutrality should be noted as evidenced by Samples Nos. 5b and 5c, 32, and 32a. Sample No. 5b, *Phoma Betae*, having a pH value of 6.5 could be quantitatively evaporated just to dryness when neutralized; when evaporated to ash

TABLE 5.

Influence of neutrality of the sample upon the stability of nitrogen during the process of evaporation.

NO.	SAMPLE ANALYZED AND METHOD OF TREATMENT (ANALYSES REPLICATED FIVE TIMES)	QUALITATIVE TESTS FOR LOSS OF NITROGEN DURING EVAPORATION	
		Diphenylamine test	Nessler test
5	*Phoma Betae*, residual solution, high in nitrate nitrogen, pH 6.5 (not adjusted to neutrality, evaporated *just to* dryness at rapid boiling)	+	—*
5a	Same as No. 5 except evaporation was less vigorous	+	—
5b	Same as No. 5 except the sample was adjusted to neutrality and evaporated *just to* dryness	—	—
5c	Same as No. 5b except the sample was evaporated to ash dryness	+	—
29	*Selaginella* extract, 14 days old, pH 5.5 (not neutralized), evaporated *just to* dryness	+	—
32	*Selaginella* extract, used immediately, pH 6.8, evaporated *just to* dryness	—	—
32a	Same as No. 32 except the sample was evaporated to ash dryness	—	—
26	*Fusarium culmorum*, residual solution plus KNO_3, pH 8.5, evaporated *just to* dryness	—	—
26a	Same as No. 26 except a trace of ammonia added, pH 9.0	—	±*
17	Greenhouse soil containing mushroom compost, water extract, pH 5.7 after 10 days standing, evaporate *just to* dryness	+	—
17a	Same as No. 17 except the extract was autoclaved for 30 minutes at 15 pounds prior to analysis	+	—
21	Sample made from same material as was No. 17, extract analyzed immediately, pH 7.1	—	—
25	*Aspergillus niger*, residual solution plus KNO_3, pH 3.9, adjusted to neutrality and evaporated *just to* dryness	—	—
25a	Same as No. 25 except evaporated to ash dryness	—	—
25b	Same as No. 25 except that the sample was *not* neutralized, pH 3.9	—	—
25c	Same as No. 25a except that the sample was *not* neutralized, pH 3.9	—	—

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* The sign + indicates positive results; the sign —, negative results; and the sign ±, that both positive and negative results were obtained.

dryness, however, there was a loss of nitrogen (Sample No. 5c). On the other hand, Sample No. 32, having a pH value of 6.8, could be evaporated to ash dryness (Sample No. 32a) without a loss of nitrogen.

Of all the samples analyzed throughout this entire investigation *Phoma Betae* was the most difficult. When the factors involved (adjustment to neutrality and evaporation just to dryness) were determined, however, no difficulty was experienced, as is shown by the quantitative results obtained (Sample No. 5, Table 4). Sample No. 25 illustrates the opposite extreme. This sample (*Aspergillus niger*), having a pH value of 3.9, required no adjustment to neutrality, even in the presence of added nitrate nitrogen. These two materials represent the extremes met with. Somewhat similar variations were observed throughout the entire list of materials used; some required adjustment to neutrality and others did not.

The method of adjusting a sample to neutrality was found to be of extreme importance to accurate procedure. The influence of this factor is apparent from a consideration of the data of Table 6.

TABLE 6.

Influence of method of neutralizing the sample upon the stability of nitrogen during the process of evaporation.

SAMPLE ANALYZED AND METHOD OF TREATMENT (all samples evaporated *just to* dryness; indicator used, brom cresol purple)	NITROGEN FOUND	QUALITATIVE TESTS FOR LOSS OF NITROGEN DURING EVAPORATION	
		Diphenylamine test	Nessler test
	mg.		
(a) Shive's nutrient solution, calculated to contain 200 mg. nitrogen per 950 cc., *indicator added to sample*, adjusted to neutrality directly....	184.3	+	–
(b) Same as (a) except *indicator was omitted*, adjusted to neutrality by adding a predetermined amount of alkali..................	202.7	–	–
(c) Shrive's nutrient solution, calculated to contain 100 mg. nitrogen per 950 cc., *indicator added to sample*, adjusted to neutrality directly...	96.3	+	–
(d) Same as (c) except *indicator was omitted*, adjusted to neutrality by adding a predetermined amount of alkali.......	101.3	–	–
(e) Shive's nutrient solution, calculated to contain 50 mg. nitrogen per 950 cc., *indicator added to sample*, adjusted to neutrality directly....	47.9	+	–
(f) Same as (e) except *indicator was omitted*, adjusted to neutrality by adding a predetermined amount of alkali..................	48.4	–	–

The data of Table 6 need little comment. The results emphasize the dangers of adding certain indicators directly to the sample in adjusting to neutrality. In every case of low quantitative results there was a corresponding positive qualitative test, indicating loss of nitrogen. In any event, no indicator that contains nitrogen can be added directly to the sample. It is recommended that when adjustment to neutrality is necessary, it be accomplished by adding a predetermined amount of weak nitrogen-free acid or alkali.

In the procedure for the modified method it is recommended that the sample be evaporated "*just to* dryness on a water bath under vacuum". It was thought desirable to investigate the factors involved in the evaporation process and to ascertain the limits of the process. Typical data obtained are given in Table 7. All the samples used contained nitrate nitrogen, because it would be meaningless and merely add to confusion to test samples that contained no nitrogen.

The outstanding fact illustrated by the data of Table 7 is that the process of evaporation cannot be carried out in any haphazard manner. Some samples require rather careful evaporation just to dryness, while other samples may be evaporated to ash dryness and heated for an hour afterward without loss of nitrogen. The most stable sample used was No. 3 (*Aspergillus niger*), which with added nitrate nitrogen (Sample No. 25) could be evaporated to ash dryness with safety. In all cases when the sample was evaporated to partial dryness only there was a subsequent loss of nitrogen when the salicylic acid mixture was added and also during the process of digestion (illustrated by Samples No. 33, 35, 37, etc.). The influence of the presence of water in the sample is demonstrated, further, by a consideration of the data for Samples No. 17, 17a, and 17b. These samples were evaporated just to dryness, and the acid mixture was added; 5 minutes later a small amount of water was added and a loss of nitrogen occurred; 24 hours later a small amount of water was added to duplicate samples, and a similar loss of nitrogen occurred. Special attention is called to Sample No. 9. This material was very high in nitrate nitrogen, second only to Sample No. 32 (*Selaginella apus*). Both of these samples (Nos. 9 and 32) required no neutralization and could be evaporated to ash dryness without a loss of nitrogen. Since the results were identical, the data for Sample No. 9 only are reported in this connection.

A special phase of the process of evaporation is illustrated by such materials as whole green plants. When the sample involved contains whole green plants considerable difficulty is experienced in evaporation under vacuum. Owing to its colloidal complex, the plant does not give up its water content rapidly in the humid atmosphere inside the flask, and the small amount of moisture present does not evolve sufficient steam to break down the plant structure effectively. Neither does the steam evolved heat the flask sufficiently to prevent condensation and consequent run-back. The balance obtained, however, is such that evaporation may be accomplished in this manner, but the time required is too long. Furthermore, it is difficult to judge, under these conditions,

TABLE 7.

Influence of the extent to which evaporation is carried upon the stability of nitrogen during the processes of evaporation, addition of the acid mixture, and subsequent digestion.

(Indicated by the relative dryness of the sample.)

NO.	DESCRIPTION OF SAMPLE (Analyses replicated 5 times)	QUALITATIVE TESTS FOR LOSS OF NITROGEN MADE					
		During evaporation		On addition of acid to sample		During digestion	
		Diphenylamine test	Nessler test	Diphenylamine test	Nessler test	Diphenylamine test	Nessler test
33	Barley plants (green) plus 10 cc. residual solution, evaporated to *partial dryness**	−	−	+	−	+	−
33a	Same as No. 33 except evaporated *just to* dryness*	−	−	−	−	−	−
33b	Same as No. 33 except evaporated to *ash dryness**	+	−	−	−	−	−
17	Greenhouse soil plus mushroom compost, water extract, evaporated just to dryness, and acid mixture was added, 5 minutes later H_2O added	−	−	+	−	+	−
17a	Same as No. 17 except H_2O added 24 hours after acid mixture had been added	−	−	+	−	+	−
17b	Same as No. 17 except evaporated to *ash dryness* and no H_2O was added later	+	−	−	−	−	−
26	*Fusarium culmorum*, residual solution plus KNO_3 solution, evaporated *just to* dryness†	−	−	−	−	−	−
26a	Same as No. 26 except evaporated to *ash dryness*†	−	−	−	−	−	−
26b	Same as No. 26 except evaporated to *partial dryness*†	−	−	+	−	+	−
9	Celery leaves and stalks, H_2O extract, evaporated *just to* dryness‡	−	−	−	−	−	−
9a	Same as No. 9 except evaporated to *ash dryness*‡	−	−	−	−	−	−

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* The same results were obtained from similar tests on the following samples: No. 35 (wheat plants plus 10 cc. residual solution), No. 37 (pea plants plus 10 cc. residual solution), No. 20 (extract of pea leaves and terminals), No. 5 (*Phoma Betae*, residual solution).

† The same results were obtained with Sample No. 25 (*Aspergillus niger*, residual solution).

‡ The same results were obtained with Sample No. 24 (mushroom compost extract plus KNO_3 solution) and Sample No. 32 (*Selaginella apus*).

when the sample is evaporated *just to* dryness (Sample No. 35). If evaporation is carried on just past this stage there is a loss of nitrogen (Sample No. 35a), especially if the sample is high in nitrate nitrogen. These difficulties may be overcome, however, (1) by adding an appreciable quantity (50–100 cc.) of distilled water, sufficient to break down completely the plant structure and organization, or (2) by using some such ventilation device as that illustrated in Fig. 4, by which dry air is used to carry off the vapors from the sample. With this device no loss of nitrogen occurred in any sample of whole green plants, even when large amounts of nitrate nitrogen were added. This latter method is recommended. Ordinary vacuum evaporation of such samples (whole green plants plus 5 to 15 cc. of residual solution) to partial dryness required 3–5 hours; just to dryness, 6–8 hours; and to ash dryness, 7–10 hours. By use of the device illustrated in Fig. 4, the time required to evaporate similar samples to comparable stages of dryness was 10–15 minutes, 12–20 minutes, and 15–25 minutes. Typical data obtained from this study are reported in Table 8.

TABLE 8.

Influence of the method of drying whole green plants upon the stability of nitrogen during evaporation, addition of the acid mixture, and subsequent digestion.*

NO.	DESCRIPTION OF SAMPLE	QUALITATIVE TESTS FOR LOSS OF NITROGEN					
		During evaporation		On addition of acid to sample		During digestion	
		Diphenylamine test	Nessler test	Diphenylamine test	Nessler test	Diphenylamine test	Nessler test
35	Wheat plants (green) plus 10 cc. residual solution high in nitrate nitrogen, *evaporated under vacuum just to dryness*.	−	−	−	−	−	−
35a	Same as No. 35 except *evaporated to full dryness under vacuum*.	+	−	−	−	−	−
35b	Same as No. 35 except *evaporated to partial dryness, vacuum*.	−	−	+	−	+	−
35c	Same as No. 35 except *evaporated just to dryness by a special ventilation* device†	−	−	−	−	−	−
35d	Same as No. 35 except *evaporated to full dryness by a special device*†	−	−	−	−	−	−
35e	Same as No. 35 except evaporated for *1 hour after sample was fully dry by a special ventilation device*†	−	−	−	−	−	−

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* The same results were obtained for barley and pea plants, Samples Nos. 33, 34, 37, 38, and for wheat, Sample No. 36.

† Reference, Fig. 4.

Practically all the various nitrogen methods contain sources of experimental error; attention to detail is essential to accurate determination. For this reason it is well to mention certain additional details of manipulation that have been found of value.

Certain grades of rubber tubing become sources of error when used to connect the condenser bulb with the block tin condenser tube of the distillation apparatus. When distillation is conducted through such connections the standard acid samples containing the distillates are more or less milky with extracted sulfides and the calculated nitrogen content is low. When such rubber tubing is subjected to repeated extractions with boiling 0.1 *N* sodium hydroxide, it will be found that a yellow-green sodium-sulfide mixture is obtained each time. It is recommended that only that rubber tubing be used which, when extracted for 30 minutes with boiling 0.1 *N* sodium hydroxide, does not yield a yellow-green mixture except during the first extraction.

In the presence of organic matter, the tendency of the digesting mixture to foam and spew out presents an irritating problem. This loss by foaming has been entirely overcome in this laboratory by allowing the acid to disintegrate thoroughly the solid portions of organic matter, without heat, over an approximate 12 hour period. It is advisable to redistribute the acid by occasional shaking. It is necessary to stopper the flasks tightly to prevent the absorption of ammonia fumes. The practice has been to add the acid and allow the mixture to stand overnight. Of all the determinations that have been made when this method was followed, not one has been lost because of foaming.

In order to determine the correct amount of alkali to use in distillation, the following test is of value: When ready to distil, add 2 drops of phenolphthalein indicator and then add the paraffin, sodium hydroxide, and zinc. After the flask is connected to the distillation apparatus and the flame is adjusted, shake the flask vigorously; if the correct amount of alkali has been added the pink color of the indicator will flash through the solution for 1 to 2 seconds and disappear. If the pink color lasts for more than 2 seconds, it is advisable to add more alkali; if the color disappears in less than 1 second, a useless excess of alkali is present. Once adjusted the amount of alkali remains practically constant so long as the amount of acid used in digestion is not varied.

The processes of evaporation under vacuum and digestion may be facilitated by adding a few small angular pieces of broken Pyrex glass. Such pieces may be used over and over again.

DISCUSSION.

In the determination of any form of nitrogen it would seem logical, first to obtain a method that is accurate for the determination of total nitrogen. The salicylic-thiosulfate method, official for fertilizers, is not accurate when applied to most biological materials in the presence of water. It is considered that the inaccuracies of this method when used for such materials have been overcome by the modifications presented in this report. The data previously mentioned and reported in the Annals of the Missouri Botanical Gardens and the data reported in this paper are offered in support of the accuracy of this modified method. The various steps in the procedure recommended for this method have been tested separately, and the limits of the processes involved have been determined. The results obtained do not warrant any alterations in the procedure as given. Certain qualifying conditions must be recognized, however: (1) Some samples must be adjusted to neutrality prior to evaporation, while other samples require no such adjustment. (2) Some samples must be carefully evaporated *just to* dryness, and other samples may be roughly evaporated *to ash* dryness without a loss of nitrogen. The particular chemical or physical complexes responsible for these differences are not known. (3) In the distillation process paraffin may be omitted in some cases, but it must be present in others to prevent foaming.

In just which cases it is safe to take advantage of these qualifying conditions in order to simplify any step in the procedure, must be decided by the individual investigator using the method. Inasmuch as these factors are essential to the accurate determination of total nitrogen in some samples they cannot be omitted from the general procedure recommended.

The above considerations lead directly to a consideration of the importance of the qualitative tests. Without some such tests it is impossible to control, accurately, the various factors involved in the quantitative determination of nitrogen by any of the acid digestion methods, impossible to know definitely that there has or has not been a loss of nitrogen in the process, and very difficult to locate any source of error that might exist. It is strongly recommended that suitable qualitative tests be considered as an integral part of nitrogen determination methods whenever it is at all feasible to use them. One or two examples, of many that could be quoted, may be given to illustrate the value of this suggestion: (1) Certain of the factors contributing to the inaccuracies of methods investigated by Mitscherlich and Herz[1] could have been definitely located and corrected in this manner. (2) Had such tests been used Strowd[2], using samples containing plant materials plus nitrate-nitrogen in *solution*, probably would have detected the inaccuracy of the "Kjeldahl method modified to include nitrate" under such conditions. (3) The use of such qualitative tests would have deterred Gallagher[3] from his theoretical denunciation of the principle of reduction of nitrates in acid medium. (4) The data given in this report show many instances in which unreliable results would have been obtained had not the quantitative data been checked with qualitative tests on the procedure used. It is fortunate, indeed, that the various methods for the determination of nitrogen are so well adapted to the use of qualitative control tests for the loss of nitrogen. It is unfortunate that they are so seldom used.

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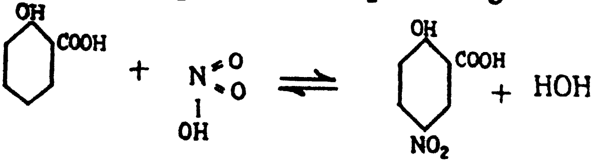
[1] *Landw. Jahrb.*, 1909, 38: 279.
[2] *Soil Science*, 1920, 10: 333.
[3] *J. Agr. Sci.*, 1923, 13: 67.

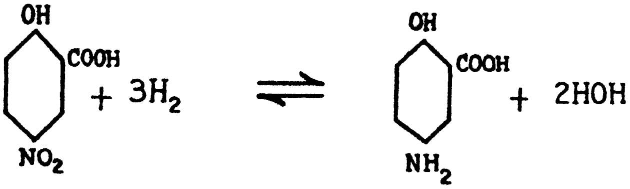
The procedure recommended for the proposed modified method is based on certain considerations of the chemical reactions involved. These reactions, so far as the nitrate-nitrogen is concerned, may be considered to take place somewhat as follows:

(1) $\overset{-}{NO_3}+\overset{++}{H_2}\ \overset{--}{SO_4} \longrightarrow \overset{+=}{HSO_4}+\overset{+-}{HNO_3}$

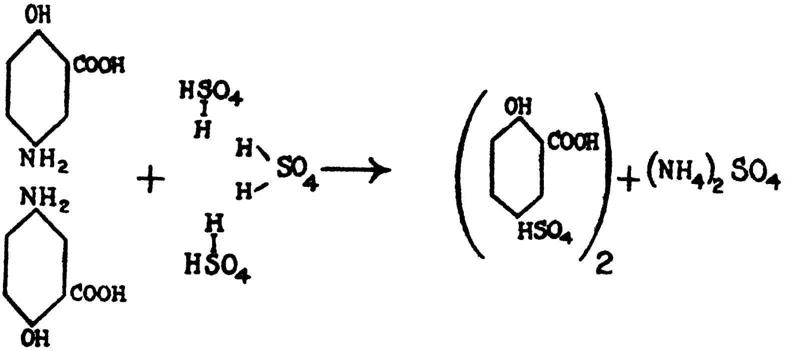
(2)



(3)



(4)



(5) $(NH_4)_2SO_4 + 2\,NaOH \rightarrow Na_2SO_4 + 2\,NH_4OH$.

Equation (3) is hastened by the addition of sodium thiosulfate, the nitro-salicylic-acid molecule being reduced (through the nitroso and hydroxylamine derivatives) to the amino compound. Ammonium sulfate may be formed by the reactions of equation (4), due to the action of sulfuric acid on the amino compound of equation (3). Other forms of organic nitrogen are reduced according to the reactions in the ordinary Kjeldahl process and will not be discussed here.

The role of sulfuric acid in the digesting mixture is three-fold:

(a) Formation of nitric acid from the nitrates present.

(b) Formation of ammonium sulfate from the amino-salicylic acid molecule.

(c) Dehydrating agent for the absorption of the 3 molecules of water formed by the reactions of equations (1) and (2), thus permitting these reactions to proceed to completion to the right.

From equation (2) it is evident that nitric acid is responsible for the nitrification of the salicylic acid molecule. The nitric acid is obtained from the nitrates present in the sample, as shown by equation (1). If water is present in the sample being determined, it is probable that the nitric acid will be diluted below the point at which it can quantitatively nitrify the salicylic acid molecule. If this condition prevails, the unstable nitric acid molecule will decompose, and loss of nitrogen dioxide gas will occur:

$$(6)\ 4\ HNO_3 \rightarrow 2\ H_2O + 4\ NO_2 + O_2.$$

In fact, in many cases when even small amounts of water are present, visible amounts of nitrogen-dioxide fumes may be given off.

Furthermore, for every molecule of ammonium sulfate formed during the process of reduction, six molecules of water are produced. Again, if water is present in the sample being determined, it is probable that the sulfuric acid will be diluted to the point where it can no longer function as a dehydrating agent as required by equations (1) and (2).

SUMMARY.

A modification of the salicylic-thiosulfate method suitable for the determination of total nitrogen in such materials as plants, plant nutritive solution, and soil extracts is given.

Results showing the accuracy of the proposed method when tested in many ways and for a wide variety of samples are reported.

Certain details of manipulation and procedure are discussed, and their influences on the accuracy of methods for nitrogen determination are demonstrated.

The value of suitable accurate qualitative tests for the loss of nitrogen, as an integral part of quantitative methods for the determination of nitrogen, is demonstrated, discussed, and recommended.

SOME INACCURACIES OF THE DEVARDA METHOD WHEN APPLIED TO PLANT MATERIALS.

By EMERY R. RANKER (Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.).

A review of the literature discloses the fact that with many plant research workers it is a common practice to estimate nitrate nitrogen by the Devarda method. No doubt the inclusion of this method in the 1925 edition of methods[1] will encourage its wider adoption for analyzing plant materials. For the estimation of nitrogen in nitrate salts it is very accurate. When applied to plant materials, however, the accuracy of the method, generally assumed, is seldom satisfactorily demonstrated under the given conditions. This is unfortunate because such application tends to bring into disrepute a method that is well suited for rapid work under certain conditions. At the Kansas City meetings of the American Association for the Advancement of Science it was stated by a plant chemist that data had been obtained indicating that approximately 75 per cent of the nitrogen content of tomato leaves was nitrate nitrogen. The Devarda method had been used in this connection, and if the data referred to be correct it will be necessary to change some of the generally accepted ideas regarding plant physiology and nitrogen metabolism. Furthermore, when this method is used on plant materials any nitrite or ammonia nitrogen present will be included in the determination, although it is known that this content of most plant materials is very low. Because of these considerations the rather preliminary investigation reported in this paper was undertaken.

MATERIALS AND METHODS.

From a wide variety of materials, the following were selected as being suitable for this study:

1. *Aspergillus niger* (whole cultures including residual solutions)	These organisms were cultured in 25 cc. of Pfeffer's solution to which 1 per cent glucose had been added. Cultures were grown until a vigorous heavy mat had formed and until all nitrate nitrogen had been used. The entire contents of 1 flask constituted 1 sample.
2. *Fusarium culmorum* (whole cultures including residual solutions)	
3. *Aspergillus niger* (residual solution only)	Cultured in 100 cc. of nutrient solution as above; samples consisted of residual solution only.
4. *Fusarium culmorum* (residual solution only)	

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 12.

10. Algae mixture, mostly *Spirogyra* (water extract)	
11. Tomato fruits, ripe (water extract)	One volume fruit and 1 volume water; extracted hot for 30 minutes.
24. Mushroom compost, KNO_3	Extracted as for No. 11, to the extract of which an equal volume of No. 74 was added.
25. *Aspergillus niger*, KNO_3	One volume No. 3 plus 1 volume No. 74.
26. *Fusarium culmorum*, KNO_3	One volume No. 4 plus 1 volume No. 74.
52. Alanine, KNO_3	One volume alanine solution (1.277 grams per liter) plus 1 volume No. 74.
53. Asparagine, KNO_3	One volume asparagine solution (0.873 grams per liter) plus 1 volume No. 74.
54. Sugar cane, KNO_3	Prepared same as No. 24.
55. Urea (2.1 grams per liter)	
57. Sugar cane	Prepared same as No. 11.
58. Sugar cane, pulp residue	Residue after extraction.
59. Mushroom compost (water extract)	One kg. dry material plus 1 liter water; stirred rapidly 4 hours; filtered.
61. Sugar beet, pulp residue	Residue after extraction.
62. Sugar beet (third water extract)	Prepared same as No. 11.
63. *Azotobacter Beijerinck* (filtrate from cultures)	Cultured in Fred's mannite solution; filtered hot, after growth.
64. Urea, KNO_3	One volume No. 55 plus 1 volume No. 74.
67. Glycine (5.0 grams per liter)	
70. Uric acid (5.0 grams per liter)	
71. Takadiastase (7.5 grams per liter)	
72. Pea seed	These seeds (Gradus variety) were germinated 5 days; mashed in a mortar to a fine pulp in 5 times their weight (dry weight) of water.
74. KNO_3 solution	This solution contained 1.443 grams of KNO_3 per liter; by analysis, 25 cc. contained sufficient nitrogen to neutralize 17.1 ±0.005 cc. of 0.02 N HCl. All the above samples, to which nitrogen was added, received 25 cc. of this solution per sample analyzed.

In selecting the above materials, with the exception of No. 74, it was necessary that they met certain specifications, namely:

(a) Freedom from nitrates or nitrites as shown by a negative diphenylamine test.

(b) Freedom from ammonia, as rendered ammonia-free, or demonstrated to be ammonia-free, by one of the three following procedures:

1. Previous alkaline distillation. The ammonia was caught in standard acid, the amount determined by titration. When this procedure was used the Devarda distillation was continued on the same sample and in the same flasks. This procedure was followed in 90 per cent of the cases.
2. Open boiling with dilute alkali for 20 minutes.
3. Negative test for ammonia with Nessler's reagent.

(An exception is noticed in Sample No. 72, which contained a trace of ammonia, probably due to amidase activity in the germination process.)

The exact procedure for the Devarda method used is as follows:

Place the sample in an 800 cc. Kjeldahl flask and add 1.0 gram of Devarda alloy for every 60–70 mg. of nitrate nitrogen present. (The reduction limit of 1.0 gram of alloy is approximately 70 mg. of nitrogen in the form of nitrate nitrogen.) Then add a small piece of paraffin, make up to a total volume of 150 cc., add 6 cc. of 10 per cent sodium hydroxide, connect to the distillation apparatus, and distil into standard acid at slow boiling for 1 hour. Titrate the standard acid to neutrality, using methyl red indicator, and calculate the nitrogen present.

Fiftieth normal acid and alkali were used in all titrations and were standardized against benzoic acid obtained from the U. S. Bureau of Standards (Sample No. 39B). The normality factors were redetermined frequently to avoid possible error from this source. Methyl red was used as the indicator, and all titrations were carried to the complete disappearance of any red tinge.

All probable errors reported in this paper were calculated according to the following formula:

$$\left(E_m = \frac{0.6745\sigma}{\sqrt{n}}\right).$$

Because the nature of the experiment demanded that the biological materials analyzed be free from nitrates, nitrites, and ammonia, none of the leaf-tissue material tested could be included. The data given are reported in terms of cc. 0.02 N hydrochloric acid neutralized. The difference between the two middle columns in Table 1 (Nitrate nitrogen present, and "Nitrate nitrogen" found) indicates what might be construed, erroneously, to be nitrate nitrogen in 15 out of the 24 samples analyzed. Previous clearing of the plant extracts did not appreciably affect these results.

Among plant chemists it is a common practice to submit the samples

EXPERIMENTAL.

The data obtained from the analyses are given in Table 1.

TABLE 1.

Non-specificity of the Devarda method for the determination of nitrate nitrogen only in various samples and especially in samples of biological materials

(Data given as cc 0.02 *N* HCl neutralized by ammonia content of distillate.)

NO	DESCRIPTION OF SAMPLE	NITRATE NITROGEN PRESENT IN SAMPLE	"NITRATE NITROGEN" FOUND BY DEVARDA DISTILLATION	NO. OF TRIALS
63	*Azotobacter Beijerinck*, filtrate	none	none	12
1	*Aspergillus niger*, whole culture	none	1.2 ± 0.02	5
2	*Fusarium culmorum*, whole culture	none	none	5
3	*Aspergillus niger*, residual solution	none	1.4 ± 0.08	6
4	*Fusarium culmorum*, residual solution	none	negligible	6
25	*Aspergillus niger*, KNO_3 solution	17.1 ± 0.01	17.1 ± 0.08	3
26	*Fusarium culmorum*, KNO_3 solution	17.1 ± 0.01	17.1 ± 0.06	3
10	Algae mixture, water extract	none	negligible	5
54	Sugar cane, KNO_3, solution	17.1 ± 0.01	27.8 ± 1.08	3
57	Sugar cane, water extract	none	16.4 ± 0.07	6
58	Sugar cane, residue, pulp	none	negligible	6
61	Sugar beet, residue, pulp	none	3.1 ± 0.04	3
62	Sugar beet, third extract	none	negligible	3
72	Pea seed germinated 5 days (mashed to fine pulp in water)	none	25.2 ± 0.59	3
24	Mushroom compost, KNO_3, solution	17.1 ± 0.01	18.2 ± 0.23	3
59	Mushroom compost, water extract	none	1.9 ± 0.32	6
11	Tomato fruits, water extract	none	3.1 ± 0.13	11
52	Alanine, KNO_3, solution	17.1 ± 0.01	17.1 ± 0.10	5
53	Asparagine, KNO_3, solution	17.1 ± 0.01	18.7 ± 0.00	5
67	Glycine, solution	none	29.9 ± 0.23	3
71	Takadiastase, solution	none	0.5 ± 0.23	3
70	Uric acid, solution	none	0.4 ± 0.15	3
55	Urea, solution	none	14.6 ± 0.73	6
64	Urea, KNO_3, solution	17.1 ± 0.01	38.0 ± 0.92	6

to a preliminary alkaline distillation without Devarda alloy. Whether or not this is done, the plant nitrogen determined by the Devarda method is quite uniformly tabulated under the heading "nitrate nitrogen". This is a misnomer and leads to misconceptions. If there be a significant correlation between such a nitrogen fraction and the physiological activity of plants, then it is suggested that the term "Devarda nitrogen" or "nitrogen by Devarda method" be used instead of the usual term "nitrate nitrogen".

CONTRIBUTED PAPERS.

THE ESTIMATION OF TERPIN HYDRATE IN TERPIN HYDRATE ELIXIR.

By A. G. Murray (Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

Having had occasion to examine a sample of terpin hydrate elixir and failing to find in the literature readily available any suitable method for the estimation of terpin hydrate, the writer decided it was necessary to devise a method.

It was known that terpin hydrate sublimes at about 100°C. and that it is volatile with steam. In order to ascertain the conditions under which a solvent could be most satisfactorily removed the following experiment was carried out:

A solution of 0.8715 gram of terpin hydrate in sufficient alcohol to make 50 cc. was prepared. Ten cc. aliquots of this solution, containing 0.1743 gram of terpin hydrate, were evaporated in different ways in weighed beakers. The weights of residues obtained were as follows:

	WEIGHT OF THE RESIDUE	ERROR
	gram	*per cent*
Evaporation on steam bath with aid of a gentle blast	0.1565	−10.3
Evaporation with aid of a gentle blast, without heat	0.1756	+ 0.8
Evaporation in vacuo without heat	0.1756	+ 0.8
Spontaneous evaporation	0.1747	+ 0.3

It was evident from the results that heat should not be used in evaporating the solvent. Because use of the blast without heat resulted in a rapid evaporation at a low temperature it was the method used in all subsequent experiments.

Terpin hydrate should not be dried in a desiccator. The U. S. Pharmacopeia states that it is "efflorescent in dry air", and it was found by experiment that the loss on drying in a desiccator overnight is quite considerable. Doubtless in a very dry climate the method of estimation herein described would not be applicable.

For the purpose of extracting terpin hydrate from the elixir it is desirable to use an immiscible solvent in which it is freely soluble. With regard to the solubility of terpin hydrate the Pharmacopeia states that 1 gram is soluble in about 200 cc. of water, 13 cc. of alcohol, 135 cc. of chloroform, and 140 cc. of ether at 25°C. The solubility data given in Squire's Companion to the British Pharmacopeia (19th ed.) are as follows: "1 in 200 of water; 1 in 14 of alcohol (90%); 1 in 46 of alcohol

(60%)”. Wallach[1] states that terpin hydrate is almost entirely insoluble in petroleum ether, but L. E. Warren[2] finds that it is slightly but appreciably soluble in petroleum ether.

Since the solubility in ether or chloroform is only slightly greater than in water, quantitative extraction with one of these solvents would probably require long continued treatment. This conclusion is apparently confirmed by Kay and Perkin[3] in a description of the synthesis of terpin, in which they speak of saturating the aqueous solution with ammonium sulfate and extracting “at least twenty times, with large quantities of ether on the shaking machine”.

It was thought that possibly acetone, which has a limited miscibility with water saturated with salt, might prove a suitable solvent. The attempt to use acetone was finally abandoned, but some of the observations made may be recorded. It was found by experiment that 1 gram of terpin hydrate dissolves in about 27 cc. of acetone at room temperature (25°C.). Terpin hydrate was not precipitated from this saturated solution in acetone by dilution with chloroform. It was found easily possible to extract terpin hydrate from the elixir by diluting with water, saturating with salt, and shaking out with a mixture of acetone and chloroform or ether, but the solvents extracted also some glycerin and sodium chloride unless the proportion of acetone in the solvent was reduced to such an extent that it no longer possessed any advantage over alcohol.

In the following method, which was found to yield the most satisfactory results, it will be noted that no account is taken of the essential oil that the official elixir contains, as the error occasioned by it is too small to be significant. If, however, it is desired to avoid the presence of the volatile oil in the residue it can be readily removed by a single preliminary extraction with a small volume of petroleum ether. The method is as follows:

Dissolve 20 grams of common salt in 100 cc. of water, or if more convenient add one volume of water to three volumes of a saturated aqueous salt solution. To a convenient measured volume of the sample of elixir add the prepared salt solution until the alcohol content is reduced to about 10 or 15 per cent by volume. Shake out with four portions, one-fourth volume each, of chloroform containing 5–7 per cent alcohol by volume. Wash each portion of the solvent successively through 5 cc. of the prepared salt solution. Filter through a pledget of purified cotton into a tared beaker or small crystallizing dish, finally rinsing the cotton and the tip of the funnel with a little alcohol. Evaporate with the aid of a blast and without the application of heat. Wipe off any moisture that may have collected on the outside of the dish and allow to stand fifteen minutes before weighing.

When 10 cc. of a sample of terpin hydrate elixir, prepared in accordance with the formula of the National Formulary with the omission of the essential oils, was assayed by the proposed method, it yielded 0.1764 gram of terpin hydrate instead of 0.1750 gram, an error of +0.8 per cent.

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[1] *Ann.*, 1885, **230**: 249.

[2] Private communication.

[3] *J. Chem. Soc. Trans.*, 1907, **91**, I: 372.

While the method was devised primarily for the assay of terpin hydrate elixir it may perhaps be adapted to any preparation of this drug that does not contain other ingredients extractable by the immiscible solvent used or that can be freed from interfering substances without loss of terpin hydrate.

THE DETERMINATION OF CASEIN IN MILK BY AN APPROXIMATELY ISO-ELECTRIC PRECIPITATION.

By Henry C. Waterman[1] (Food Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

A discussion of the desirability of approximating the iso-electric point in the quantitative precipitation of casein from milk together with an outline of a possible method has been published by the writer of this paper[2]. A further study of milk protein precipitation, however, has suggested a method shorter than that previously outlined and also more accurate with regard to pH adjustment. The revised method is given here as it was developed.

FIXED VOLUME PRECIPITATION.

When equal volumes of various milks are treated with a fixed volume of an acid having physical properties optimal for buffering at the desired pH value, a close agreement among the pH values of filtrates is not obtained. Buffering salts are formed, but they are formed from milk constituents that vary not only in total, but in relative quantity. It was to overcome this irregularity that the titration procedure[1], specifying a diluted highly ionized acid to minimize the buffer action and an indicator for the determination of the correct end point, was formerly suggested.

It was found, however, that fixed volume precipitation with adjustment by buffer action could be made simpler, more accurate, and more rapid than the titration-controlled precipitation. Instead of permitting the buffer-forming acid to produce indeterminate mixtures of salts from the varying constituents of the milks, the acid is combined in the reagent itself with a definite, adjusted concentration of one of its alkali salts to form a buffer mixture of rather high total concentration. A fixed volume of such a reagent precipitates casein from fresh milks within the quite satisfactory variation range of about 0.04 pH, and this range may be placed very near the iso-electric point.

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[1] The writer desires to express his gratitude to the Leeds and Northrup Co., Philadelphia, whose loan of the necessary potentiometric apparatus made it possible to perform the preliminary experiments and to obtain the data of Table 1 at the Exhibit Laboratory of the Bureau of Chemistry at the Sesquicentennial International Exposition, Philadelphia, 1926.

[2] *This Journal*, 1926, 9: 246.

The gain in rapidity and convenience is evident. A less obvious advantage lies in the fact that the reagent need have but little higher active acidity than is required in the actual precipitation. Strong local over-acidification in adding the precipitant, which tends to an irreversible precipitation (denaturation) of some non-casein milk protein, is thus rendered impossible. The pH of the precipitant here proposed, for example, is only 0.12 to 0.15 pH below the precipitation pH value. Finally, the volume of the buffer precipitant required for a given volume of milk is much larger than is that of the single acid. This makes measurement of the precipitant easier and more accurate; and, as shown by the starred figures in the last column of Table 3, the nature of the proposed reagent also renders very accurate measurement unnecessary.

SELECTION OF pH VALUE FOR THE PRECIPITATION.

A number of somewhat variant figures have been given as the iso-electric point of casein. The recently published figure (pH 4.85) of Csonka, Murphy, and Jones[1] seemed applicable since it represents the point of least solubility of casein in buffer mixtures. Rona and Michaelis[2] place the value at pH 4.74, however; and, according to the first named authors, some still lower values, about pH 4.6, are also to be found in the literature.

Preliminary experiments were made at pH 4.85. It was found, however, that at this pH value some of the protein was precipitated in a finely divided condition inconvenient for filtration. As a slightly lower pH value seemed likely to improve filtrability, an approximation of the figure (pH 4.7) used by Bleyer and Kallmann[3] in a recent and elaborate study of the nitrogenous constituents of milk was attempted. Extreme simplicity of procedure in making up the reagent was also considered important, however, and in securing this an average precipitation pH of 4.73, almost identical with the iso-electric point according to Rona and Michaelis, above cited, was obtained. The agglomeration of the precipitate was as satisfactory as could be expected in a mixture so near in pH value to the iso-electric point of the protein.

THE PRECIPITANT.

Maximum buffer effect at a given pH requires an acid having an ionization exponent (pK_a) numerically very nearly equal to the given pH, with an appropriate admixture of one of its alkali salts[4]. Equation of pH to pK_a does not occur precisely at the mid-point of the titration curve, but the approximation is ample for the present purpose. To make a buffer precipitant for casein, then, an acid of pK_a 4.7 was needed. Acetic acid has exactly this value. Experiment, therefore, was confined to mixtures of acetic acid and sodium acetate. The very simply prepared equimolecular mixture was found satisfactory; under the conditions prescribed in the proposed method, it gave closely concordant adjustments (Tables 1 and 3). This mixture also possesses the advantage of a well established pH value, having been studied by Michaelis[1] and by Walpole[2], both of whom used it as a checking standard for electrometric apparatus, and regarded its electrode potential as reproducible with great accuracy.

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[1] *J. Am. Chem. Soc.*, 1926, 48: 763.

[2] *Biochem. Z.*, 1910, 28: 193.

[3] *Ibid.*, 1924, 153: 459.

[4] Clark, W. M. The Determination of Hydrogen Ions, 2nd ed., p. 25. Baltimore, Williams & Wilkins Co., 1925. Hober, Rudolf Physikalische Chemie der Zelle und der Gewebe, 6th ed. (rev.), p. 100. Leipzig, Wilh Engelmann, 1926.

DETERMINATION OF THE PRECIPITATED NITROGEN.

Casein cannot be washed with water, as directed in the official methods[3] of the Association of Official Agricultural Chemists, without losing much of the effect of any pH adjustment made in its precipitation. An adjusted washing solution would add undesirable complication; and the time required for a thorough washing of a protein precipitate was regarded as prohibitory. The precipitated nitrogen was therefore determined as the difference between total milk nitrogen and nonprecipitable nitrogen. These two nitrogen determinations required no more time than did single determinations, on precipitate with filter paper, in the official casein methods. The washing required in the official method is tedious, particularly toward the end, and the filter paper greatly delays clearing in Kjeldahl digestion, causing troublesome frothing and charring. In the proposed method, the nitrogen samples of both the milk and the casein-free filtrate clear rapidly and without foaming.

TIME REQUIREMENT, OFFICIAL vs. PROPOSED METHOD.

The proposed method is believed the shorter and more convenient procedure. The writer completes analyses by the proposed method in from 1 to 1½ hours less time than by Official Method I.

PROPOSED METHOD.

REAGENT.

Pipet 250 cc. of normal acetic acid into a 1000 cc. flask. Add 125 cc. of normal, carbon-dioxide-free sodium hydroxide. Make up to 1000 cc. with carbon-dioxide-free distilled water and mix thoroughly.

DETERMINATION.

Pipet 20 cc. of the sample into a 100 cc. flask. Add 50 cc. of the reagent, mix, make up to volume with distilled water, and shake well. Set the flask in hot water (50°–60°C., *not over* 60°C.) and let stand 15 minutes. Cool to room temperature and filter. Use a double folded paper, returning the filtrate once or twice to the filter; then filter once through a hardened paper. Determine nitrogen (A) in 50 cc. of the clear filtrate, and determine total nitrogen (B) in 10 cc. of the milk. 6.38 × (B − A) = casein in 10 cc. of the milk. Report grams of casein per 100 cc. of milk, or divide the grams per 100 cc. by the density of the milk, and report as percentage by weight.

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[1] Die Wasserstoffionenkonzentration, Berlin, 1914.
[2] *J. Chem. Soc.*, 1914, **105**: 2501, 2521
[3] *Methods of Analysis, A. O. A. C.*, 1925, 260.

SUMMARY.

A method is proposed for the determination of casein in milk, wherein a buffer mixture, used as the precipitant, controls the pH of precipitation

TABLE 1.

Precipitation pH values with various samples by proposed method for casein in milk.

SAMPLE NO.	MILK SAMPLE		PRECIPITANT		PRECIPITATION pH VALUE	
	pH Value	Volume Taken	pH Value	Volume Taken	pH Filtrate from Casein	Measurement Temperature
		cc.		*cc.*		*°C.*
5 Fresh	6.62	20.0	4.61	50.0	4.73	19
6-a Fresh	6.65	20.0	4.61	50.0	4.73	20
6-b Stale, but not curdled	5.86	20.0	4.61	50.0	4.69	18
6-b Stale, but not curdled	5.86	20.0	4.61	50.0	4.68	18
7-a Fresh	6.70	20.0	4.61	50.0	4.73	22
7-a Fresh	6.70	20.0	4.61	50.0	4.73	22
7-b Stale, sour odor, but not curdled	5.39	20.0	4.61	50.0	4.68	21
7-b Stale, sour odor, but not curdled	5.39	20.0	4.61	50.0	4.68	21
8 Fresh	6.73	20.0	4.61	50.0	4.74	22
8 Fresh	6.73	20.0	4.61	50.0	4.74	22
10 Fresh	6.65	20.0	4.61	50.0	4.75	19
10 Fresh	6.65	20.0	4.61	50.0	4.75	19
	Maximum Difference 1.34 pH unit				*Maximum Difference* 0.07 pH unit	

within a range of about 0.04 for fresh milk samples. Precipitation is made at very nearly the iso-electric point of casein. Casein nitrogen is determined as the difference between total milk nitrogen and nonprecipitable nitrogen, the necessity for attempting to wash casein precipitates quantitatively being thereby avoided. The results quoted are more consistent than those obtained in parallel trials in which Official Method I of the Association of Official Agricultural Chemists was used. Data are submitted showing that by the Official Method casein is precipitated at varying pH values much lower than the iso-electric point of the protein.

TABLE 2.

Precipitation pH values with various samples by Official Method I for casein in milk.

(Volume taken: 10 cc ; precipitant: 1.5 cc. 1 + 9 acetic acid)

MILK SAMPLE		PRECIPITATION pH VALUE	
No.	pH Value	pH Filtrate from Casein	Measurement Temperature °C.
9	6.72	4.13	20
9	6.72	4.13	20
10	6.65	4.20	18
10	6.65	4.21	18
11-b Slightly stale	6.34	4.09	26
11-b Slightly stale	6.34	4.14	26

TABLE 3.

Analytical results for casein in milk by official and proposed methods.

SAMPLE NO.	pH Value	OFFICIAL METHOD		PROPOSED METHOD	
		Casein (gram/100 cc.)	pH Value Filtrate	Casein (gram/100 cc)	pH Value Filtrate
12	6.63	2.70	4.15	2.64	4.74
12	6.63	2.64	4.16	2.64	4.73*
12	6.63	2.64	4.15	2.68	4.74
12	6.63	2.41	4.15	2.62	4.75*
12	6.63	Average 2.60 Max. Diff. 0.29	Average 4.15	Average 2.65 Max. Diff. 0.06	Average 4.74
13	6.67	3.23	4.31*	2.87	4.77
13	6.67	2.92	4.20*	2.94	4.77
13	6.67	2.96	4.22*	2.92	4.77
13	6.67	Average 3.04 Max. Diff. 0.31	Average 4.24	Average 2.91 Max. Diff. 0.07	Average 4.77

* Precipitant measured from graduated cylinder of smallest sufficient capacity. Figures not starred represent pipet measurement of precipitant.

APPLICATION OF THE STAHRE REACTION TO THE ACCURATE DETERMINATION OF CITRIC ACID.

By B. G. HARTMANN and F. HILLIG (Food Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

The Stahre reaction[1] for the qualitative determination of citric acid as pentabromacetone[2] has been studied by a number of investigators. Wöhlk[3], after a close study, concluded "that the reaction is not quantitative; that the dilution, the temperature and the quantity of bromine are determining factors for quantitative recoveries of pentabromacetone". Kunz[4] demonstrated that by substituting potassium bromide for bromine water, the reaction could be utilized for the quantitative determination of the acid. He also showed that tartaric and malic acids do not interfere with the determination. Dunbar and Lepper[5] made a collaborative study of this method for the determination of citric acid and found that with large quantities of acid the Kunz procedure gave acceptable results. No work is reported on small quantities of the acid. It was upon the recommendation of Dunbar and Lepper that the method was adopted by the Association of Official Agricultural Chemists[6] as a tentative method for the determination of citric acid in fruit products.

Analysts have experienced some difficulty with the determination, particularly when the quantity of citric acid present in the aliquot under examination was small, and this condition has been recognized in the text of the tentative method inasmuch as a minimum acid content of 50 mg. is specified.

D. H. Tilden and H. J. Wichmann[7] reported a series of results obtained by the tentative method on varying quantities of citric acid in aqueous solution. They presented data showing that the loss of citric acid varies almost directly with the dilutions, and also that a quantity less than 100 mg. in the aliquot under examination yields unsatisfactory results.

The poor results obtained, it is believed, are attributable to the fact that the conditions for the formation of pentabromacetone and the manner of collecting and washing the precipitate are not definitely controlled, and not to any fault of the principles of the reaction itself. Furthermore, the text regarding the oxidation with potassium permanganate is rather ambiguous.

Pentabromacetone is a well-defined crystallizable compound with a melting point of 73°C. It is readily soluble in alcohol and ether but only sparingly soluble in dilute sulfuric acid. Because of its relatively low melting point, it tends to separate during the bromination-oxidation procedure as oil, which, unless it is thoroughly chilled, crystallizes only with difficulty. Obviously, failure to crystallize the oil formed will result in a loss. It is necessary to guard against loss during drying because pentabromacetone is volatile at room temperature. The method prescribes drying in vacuo over sulfuric acid. It has been the experience of the authors, however, that drying overnight in vacuo over sulfuric acid is not always sufficient to insure complete removal of moisture. Pentabromacetone in the dried state is not appreciably volatile at ordinary temperatures under atmospheric pressure. There is, however, no assurance that in a moist state under reduced pressure it is not measurably volatile.

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[1] This well-known reaction comprises the oxidation of citric acid into acetone and the substitution of bromine by means of bromine water to form pentabromacetone.

[2] *Nord. Farm. Tidskrift.*, 1895, 2: 141.

[3] *Z. anal. Chem.*, 1902, 41: 77.

[4] *Arch. Chem. Mikrosk.*, 1914, 7: 285.

[5] *This Journal*, 1920, 3: 402.

[6] *Methods of Analysis, A. O. A. C.*, 1925, 215

[7] Unpublished report of the U. S. Food and Drug Inspection Station, San Francisco, Calif.

It should be apparent, therefore, that the physical properties of pentabromacetone are not conducive to the production of quantitative yields unless each step in the reaction is carefully controlled. Notwithstanding this fact, the experience of the writers with the method shows that with proper precautions it should be possible to overcome these difficulties without altering the general principles underlying the procedure indicated by Kunz. The investigational work described in this paper fully substantiated this belief.

EXPERIMENTAL.

First it seemed desirable to determine whether the two main reactions involved in the formation of pentabromacetone (oxidation of citric acid to acetone dicarboxylic acid and substitution of bromine) are truly quantitative. To do this, it seemed necessary to suppress the solubility of pentabromacetone as effectively as possible and to accomplish the drying in the least possible time.

The results presented in Table 1 were obtained on solutions containing 92.9 mg. of anhydrous citric acid. These solutions were prepared by adding to 10 cc. of a standard solution of citric acid containing 92.9 mg. of anhydrous citric acid, dilute sulfuric acid (1 + 1) in the proportion of 5 cc. to each 50 cc. of the final volume, 10 cc. of saturated bromine water, 5 cc. of a 27 per cent solution of potassium bromide, and sufficient water to complete the volumes recorded in the second column of the table.

The mixtures were heated to 48°–50°C. for 5 minutes and immediately treated with 15 cc. of a 5 per cent potassium permanganate solution. The potassium permanganate solution was added, under constant agitation, in 2 cc. portions at intervals of 10 seconds. It was found that this quantity of permanganate was sufficient to give the supernatant liquid the brownish color that Stahre recommends. After permitting the reaction mixture to stand for 1 minute, 40 cc. of a 20 per cent ferrous sulfate solution was added from a buret, drop by drop, to dissolve the precipitated manganese dioxide. The mixture was then cooled in ice water and, after vigorous shaking, placed in a refrigerator. After several hours in the refrigerator it was again vigorously shaken and allowed to stand overnight. The pentabromacetone was transferred onto a thin pad of asbestos in a Gooch crucible by means of gentle suction, and because it showed a tendency to cling to the sides of the flask it was necessary to use a rubber-tipped glass rod to insure complete removal of the precipitate. To obviate possible loss owing to the large quantities of wash solutions required the precipitate was transferred to the crucible by means of the filtrate. After the precipitate had been collected, it was washed with 50 cc. of cold diluted sulfuric acid (1 + 100) and finally with 50 cc. of cold water. The pentabromacetone was then dried in a current of dry air and immediately weighed. The factor 0.424 was used for computing citric acid, anhydrous.

TABLE 1.

Influence of dilution on the determination of citric acid.

(Citric acid (anhydrous) present, 92.9 mg.)

SOLUTION	VOLUME	FOUND	AVERAGE	LOST	AVERAGE	FOUND
	cc.	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
1	50	91.1		1.8		
1	50	90.4	90.8	2.5	2.1	97.7
2	75	90.7		2.2		
2	75	90.1	90.4	2.8	2.5	97.3
3	100	89.9		3.0		
3	100	90.5	90.2	2.4	2.7	97.1
4	125	89.9		3.0		
4	125	89.1	89.5	3.8	3.4	96.3
5	150	89.3		3.6		
5	150	88.7	89.0	4.2	3.9	95.8
6	200	87.9		5.0		
6	200	87.9	87.9	5.0	5.0	94.6

The results recorded in Table 1 show good agreement of duplicates, and the percentage of citric acid found for the various dilutions is high. There is a gradual increase in loss of acid as the dilutions increase. Using the final volumes of the various mixtures from which the pentabromacetone was recovered (volume indicated in the second column of the table plus 55 cc.), an average loss of citric acid of 1.9 mg. per 100 cc. reaction mixture is shown. That the losses of citric acid recorded in the table are due to solubility of pentabromacetone in the reaction mixtures was demonstrated by the fact that a small quantity of pentabromacetone was obtained when the filtrate was extracted with ether.

SENSITIVITY OF STAHRE REACTION.

The next step in the study of the method was directed to the determination of the sensitivity of the Stahre reaction. In Table 2 results obtained with solutions adjusted to 100 cc. and containing varying quantities of pure anhydrous citric acid are presented.

TABLE 2.

Effect of solubility of pentabromacetone on determination of citric acid.

SOLUTION	PRESENT	FOUND	LOST	FOUND
	mg	*mg*	*mg*	*per cent*
1	4.7	1.8	2.9	38.3
2	11.8	8.2	3.6	69.5
3	23.5	19.2	4.3	81.7
4	93.2	90.8	2.4	97.4

With the smaller quantities of citric acid the percentages found are low, but the losses for the four solutions are very similar (average 3.3 mg.). In these experiments the loss of citric acid through solubility of pentabromacetone is 2.1 mg. per 100 cc. of reaction mixture.

Having established that the conversion of citric acid into pentabromacetone is practically quantitative, the writers next attempted to adjust the procedure to working conditions that would permit of the highest possible results to be obtained in the determination. To do this it seemed necessary to provide means for (1) preventing the formation of pentabromacetone as the oil, (2) suppressing the solubility of pentabromacetone to a minimum, and (3) drying the precipitate for weighing.

1. *Preventing the formation of pentabromacetone as the oil.*

To overcome the tendency to form an oil that is not readily crystallizable and that for this reason might cause a loss of an appreciable quantity of pentabromacetone, thorough chilling of the oxidation mixture before adding the ferrous sulfate solution was tried. It was thought that in this way the formation of oil might be greatly suppressed if not entirely avoided. Experiments in this direction showed that an actual gain in the percentage of citric acid found occurred when such a procedure was followed. This is illustrated in Table 3.

TABLE 3.

Effect of cooling on the determination of citric acid.

SOLUTION	PRESENT	FOUND, NOT COOLED	FOUND, COOLED
	mg	*per cent*	*per cent*
1	4.7	38.3	47.4
2	23.5	81.7	85.8
3	93.2	97.4	97.9

The formation of oil and its subsequent crystallization produce a material that clings tenaciously to the sides of the flask. This is not noticeable, however, when the pentabromacetone crystallizes without going through the oil stage. It was believed that a small quantity of asbestos would prevent this clinging of the pentabromacetone to the glass surface. Experiments established the fact that the addition of asbestos is not only helpful, but that its use slightly improves the yield.

2. *Suppressing the solubility of pentabromacetone.*

In order to prevent loss of precipitate through its reversion to the oil condition, chilled solutions for washing were employed. Tightly tamped felts of asbestos were used in the Gooch crucibles. Since the drying was accomplished by means of dry air, it was found advantageous to use as thin a felt as possible. The supernatant liquid, which is generally clear, was poured into the Gooch crucible, and the filtrate obtained was used for transferring the precipitate onto the pad. The precipitate was then washed with 50 cc. of ice-cold sulfuric acid (1 + 100) in three portions, being sucked dry after each addition. The contents of the crucible were then washed with 50 cc. of ice-cold water in three portions.

3. *Drying the precipitate for weighing.*

The procedure for drying pentabromacetone in vacuo over sulfuric acid is not always dependable and is time-consuming. It was found that the precipitate could be dried in less than one-half hour by aspirating it in a current of dry air. In fact, in all the determinations recorded in this investigation the pentabromacetone was dried in this manner. Some precautions, however, must be observed in order to obtain the most satisfactory results. It was found that violent aspiration had a tendency to yield slightly low results, while no trouble was experienced in obtaining constant weight to within 0.1 to 0.3 mg. within one-half hour when the dried air was applied under proper conditions. For the purpose of drying and purifying the air it was passed through sulfuric acid, soda lime, and a cotton filter. The seal between the drying apparatus and the Gooch crucible was made by clamping tightly on the cup a large rubber stopper carrying a perforated delivery tube. It was found that the tendency of pentabromacetone to creep would not permit fitting a rubber stopper into the crucible. The intake tube for the air was also provided with small perforations through which the air was sprayed into the sulfuric acid. The side perforations in the delivery tube allowed equal distribution of dry air to all parts of the crucible.

OXIDATION WITH PERMANGANATE.

Thus far in this paper no reference has been made to the oxidation of citric acid with permanganate with the exception of the statement that the text of the tentative method regarding this operation is decidedly ambiguous. In fact, the writers were not able to follow the directions with any assurance. In the course of experiments to formulate more exacting conditions for the oxidation, it developed that splendid yields could be obtained by permitting the permanganate reaction to proceed "en masse" instead of by gradual steps, as directed in the procedure of the tentative method. It also developed that an excess of permanganate is not harmful to the reaction. The quantity of permanganate required, is, of course, dependent upon the quantity of oxidizable material present in the sample under examination. From the work done in this investigation on pure citric acid, it may be stated that a distinct brown coloration of the liquid overlying the manganese dioxide precipitate marks the completion of the reaction.

WEIGHING THE PENTABROMACETONE.

In the course of experiments on the weighing of small quantities of pentabromacetone, it was found that better agreement could be obtained by removing the precipitate from the Gooch crucible with alcohol and ether and ascertaining the weight by difference than could be obtained by weighing direct. Pentabromacetone is so readily soluble in alcohol and ether that such a procedure offers no difficulties. Three applications of 20 cc. each of alcohol, followed by three portions of 20 cc. each of ether, will completely remove several decigrams of pentabromacetone from the crucible. The solvent should remain in contact with the material for a short time after each application before applying suction.

THE FUNCTION OF BROMINE WATER.

The role of bromine water in the determination was investigated, but it was found that its use is not necessary for completing the Stahre reaction. Experiments with and without the addition of bromine water showed no difference in the yield of pentabromacetone. Kunz found that in the determination of citric acid in wine a precipitate with bromine was obtained which was not due to pentabromacetone. Accordingly, he recommended the addition of bromine water and filtration before brominating with potassium bromide.

THE ACTION OF OTHER FRUIT ACIDS.

The observation by Kunz that other fruit acids do not interfere with the determination of citric acid was substantiated by the writers. A solution (100 cc.) containing 50 mg. each of tartaric, malic, oxalic, and benzoic acids was treated according to the procedure described at the conclusion of this paper. No pentabromacetone was recovered. An addition of 54.8 mg. of anhydrous citric acid to another portion of the solution gave in duplicate 50.3 and 50.1 mg., or 53.0 and 52.8 mg. when corrected for solubility of pentabromacetone. Additional experiments were made on the behavior of iso-citric acid when treated by the pentabromacetone procedure. In a study of the non-volatile acids of the blackberry, E. K. Nelson[1] showed that the predominating and characteristic acid is optically active iso-citric acid. Therefore, an aqueous solution of the pure triethylester of the acid containing 108 mg. per 100 cc. was, after saponification, treated by the procedure described at the conclusion of this paper. No pentabromacetone was recovered. From these experiments it is apparent that the organic acids ordinarily found in fruits do not interfere with the Stahre reaction.

With the information that this investigational work afforded, a modified procedure for the determination of citric acid by the pentabromacetone method was worked out. This procedure is given at the conclusion of this paper, and by it the results shown in Columns B of Table 4 were obtained. Columns A and C are included in the table to show by comparison the results obtained after 2 hours' cooling in the refrigerator instead of overnight and the effect that a gradual oxidation with potassium permanganate has upon the completion of the reaction by which pentabromacetone is formed. The volumes of the solutions before bromination were held to 100 cc., totaling 155 cc. for the final reaction mixtures, in each instance.

TABLE 4.

Determination of citric acid under controlled conditions.

PRESENT	TWO HOURS IN REFRIGERATOR	OVERNIGHT IN REFRIGERATOR	SLOW ADDITION OF PERMANGANATE. IN REFRIGERATOR OVERNIGHT	LOST			FOUND		
	A	B	C	A	B	C	A	B	C
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*	*per cent*
4.5	0.7	2.3 2.1 Ave. 2.2	2.2	3.8	2.3	2.3	15.6	48.9	48.9
11.2	8.3	8.8 8.7 Ave. 8.8	8.8	2.9	2.4	2.4	74.1	78.6	78.6
22.5	19.5	19.6 20.1 Ave. 19.9	19.8	3.0	2.6	2.7	86.7	88.4	88.0
45.2	41.6	42.3 43.9*	42.6	3.6	2.9	2.6	92.0	93.6	94.3
90.3	85.9	86.5 87.0 Ave. 86.8	87.8	4.4	3.5	2.5	95.1	96.1	97.2
			Ave.	3.5	2.7	2.5			

* Not used in further calculations. Constant weight could not be obtained.

J. Am. Chem. Soc., 1925, 47: 568.

From the data given in Columns B of Table 4 it is evident that the modified method, in which each step of the procedure is carefully controlled, will give high yields of pentabromacetone. Even with a quantity of citric acid so small as 4.5 mg. the quantity of pentabromacetone is such that after calculating its equivalent of citric acid and making allowance for the loss through solubility of the pentabromacetone in the reaction mixture the result is not very far from the truth. The quantity of pentabromacetone soluble in each 100 cc. of the reaction mixture from which it is crystallized is shown by a simple calculation to be equivalent to 1.7 mg. of anhydrous citric acid. The results in Columns C show that the gradual addition of the permanganate gives little if any better results than when the entire amount of permanganate solution required is added at one time. A comparison of the data reported in Columns A and B shows that long standing in the cold (refrigerator) is necessary to obtain a maximum yield of pentabromacetone.

From these results it is concluded that the modified Stahre reaction for the conversion of citric acid into pentabromacetone is not only quantitative, but that it furnishes a splendid means for accurately determining the acid. The procedure for the determination of citric acid formulated as a result of the work is as follows.

PROCEDURE FOR THE DETERMINATION OF CITRIC ACID AS PENTABROMACETONE.

REAGENTS.

Potassium bromide solution Dissolve 15 grams of potassium bromide in 40 cc of water.

Potassium permanganate solution —Dissolve 5 grams of potassium permanganate in water and dilute to 100 cc.

Ferrous sulfate solution.—Dissolve 20 grams of ferrous sulfate in 100 cc of water containing 1 cc. of concentrated sulfuric acid

Bromine water —Freshly prepared saturated solution

Asbestos -Treat asbestos of the amphibole variety as directed in the official methods[1]. Purify further by permitting it to be acted upon by the reagents used in the determination of citric acid and in a manner analogous to that used in the actual determination.

DETERMINATION.

To 100 cc. of the citric acid solution add exactly 10 cc each of dilute sulfuric acid (1 + 1) and bromine water. Allow to stand 10 minutes If a precipitate is formed, filter. To 100 cc. of the filtered or unfiltered solution, add 5 cc of the potassium bromide solution and about 0.3 gram of the purified asbestos. Heat the mixture to 48°-50°C and maintain this temperature for five minutes Now add at once and all at one time 15 cc. of the potassium permanganate solution and allow to stand for 10 minutes shaking occasionally. If the liquid overlying the separated manganese dioxide is not colored brown, add more potassium permanganate solution Cool the mixture in ice water and add 40 cc. of ice-cold ferrous sulfate solution. If the manganese dioxide has not been completely dissolved, add more ferrous sulfate solution. Note the volumes of the various solutions added. Shake for 5 minutes, cooling in ice water occasionally to keep the mixture thoroughly chilled. Place in a refrigerator overnight. Filter by decantation onto a thin, tightly tamped pad of asbestos in a Gooch crucible. Transfer the contents of the flask to the crucible with the filtrate. (It is important that the filtering operation be completed as quickly as possible.) Now wash the contents of the crucible at once with three portions of 20 cc. each of ice-cold sulfuric acid (1 + 100) and three portions of 20 cc. each of ice-cold water. Immediately dry the precipitate by aspirating with dry air until constant weight to within 0.3 mg. is obtained. Dry the air by passing it through sulfuric acid and soda lime and finally filter through cotton. Provide an intake tube for the air and a delivery tube to connect with the Gooch crucible that have small perforations. Suck the air through the system at a slow rate. For a seal between the drying apparatus and the cup of the crucible, clamp onto the cup a large rubber stopper carrying the air delivery tube. Hold the stopper in place by means of a ring clamped to a ring support. If the drying can not be undertaken at once, place the crucible in the refrigerator. To remove the pentabromacetone treat the contents of the crucible with three portions of 20 cc. each of alcohol and three portions of 20 cc. each of ether. Aspirate with dry air to constant weight and reweigh. Multiply the difference in the weights by 0.424 to obtain the grams of anhydrous citric acid in the aliquot taken. Correct for the solubility of pentabromacetone by adding to the citric acid thus obtained 1.7 mg. for each 100 cc. of the reaction mixture.

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[1] *Methods of Analysis, A O A C*, 1925, 190

A COMPARISON OF SEVERAL PROCESSES FOR THE ASSAY OF PODOPHYLLUM[1].

By L. E. WARREN (Drug Control Laboratory, Bureau of Chemistry, Washington, D. C.).

Having had occasion to assay a specimen of podophyllum for its resin content, the writer tried the process described in U. S. Pharmacopeia X. The drug tested was furnished by The Wm. S. Merrell Company, with the statement that in the preparation of the resin on a commercial scale large batches of this particular lot of drug had yielded an average of almost exactly 5 per cent of podophyllin.

The assay for podophyllum in the U. S. P. X is the same as that for jalap. For convenience of reference it is given herewith as applied to podophyllum:

ASSAY OF PODOPHYLLUM BY U. S. P. X, METHOD I.

Place 10 grams of podophyllum, finely powdered, in a dry flask of about 200 cc. capacity, add 50 cc. of alcohol, and stopper the flask with a perforated cork holding a reflux condenser. (An open glass tube of not less than 24 inches in length will suffice.) Place the flask on a water bath, and digest for 3 hours with occasional shaking. Then transfer the drug to a small percolator, allow it to drain, and percolate with alcohol until the percolate measures 100 cc. Allow to cool to room temperature, and add alcohol to make exactly 100 cc. Mix well.

Transfer 20 cc. of this tincture, accurately measured, representing 2 grams of podophyllum, to a separator, and add 10 cc. of chloroform and 20 cc. of a saturated solution of potassium citrate (20 grams of potassium citrate dissolved in 12 cc. of distilled water). Shake well during 2 minutes, then set aside for not less than 10 hours or overnight. Draw off and discard the lower aqueous liquid, and filter the alcohol-chloroform solution through a small filter, wetted with alcohol-chloroform, into a tared flask or beaker. Rinse the separator with a mixture of 10 cc. of alcohol and 5 cc. of chloroform, and pass the rinsing through the filter. Mix the chloroformic liquids, evaporate the solution on a water bath, dry the residue at 100°C., and weigh.

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[1] Read before the Division of Medicinal Products, American Chemical Society, at Richmond, Va. April 14, 1927. Published through courtesy of *Industrial and Engineering Chemistry*.

The results obtained by the U. S. P. X assay were, respectively, 6.99, 6.83, and 7.14 per cent of resin. Since these results were considerably higher than those usually obtained from good grades of authentic podophyllum and were much higher than was expected from this particular specimen, it was decided to assay the specimen by the U. S. P. IX process and by the Jenkins method[1]. The process given for the assay of podophyllum in the U. S. P. IX is not definitely described. The method is that directed for the production of resin of podophyllum on a commercial scale. For the purpose of adapting the method to small quantities it was rewritten as follows:

ASSAY OF PODOPHYLLUM, METHOD II.

(Adaptation from U. S. P. IX.)

Moisten 10 grams of podophyllum in a No. 60 powder with 5 cc. of alcohol and pack it in a cylindrical percolator; then add enough alcohol to saturate the powder and leave a stratum above it. When the liquid begins to drop from the percolator, close the lower orifice, and, having closely covered the percolator, macerate for 48 hours. Then allow the percolation to proceed, gradually adding alcohol, until the percolate ceases to produce more than a slight turbidity when dropped into water. Evaporate the alcohol in a tared beaker until the percolate is reduced to the consistence of a thin sirup, and pour this slowly, with constant stirring, into a second tared beaker containing 10 cc. of water previously mixed with 1 cc. of normal hydrochloric acid and cooled to a temperature below 10°C. When the precipitate has subsided, decant the supernatant liquid into a tared Gooch crucible and wash the precipitate in the beaker twice by decantation with fresh portions of 5 cc. each of cold water slightly acidulated with hydrochloric acid. Transfer the precipitate to the crucible by means of small portions of cold water slightly acidulated with hydrochloric acid. Dry the contents of the crucible at 100°C. and weigh. If particles of resin adhere, dry the beakers and their contents at 100° C., cool, weigh, and add the total net weight to the weight of the crucible contents.

The Jenkins method is essentially as follows:

ASSAY OF PODOPHYLLUM, METHOD III.

Place 10 grams of the drug in a No. 60 powder in an Erlenmeyer flask of about 200 cc. capacity and add 25 cc. of alcohol. Fit the flask with a stopper through which is inserted a glass tube about 24 inches in length to act as a condenser, and leave the flask on a sand bath at 80°C. for 3 hours. Transfer the contents of the flask to a small percolator and wash with alcohol until about 50 cc. of percolate is obtained. Cool to room temperature and make up the solution to exactly 50 cc. Use 10 cc. of this solution, representing 2 grams of the drug, for each assay.

Measure 10 cc. of the tincture of podophyllum prepared as above described into a separator and add 10 cc. of chloroform and 10 cc. of acidulated water containing 0.6 per cent of hydrochloric acid (2 cc. of hydrochloric acid in 100 cc. of water). Shake the mixture and allow it to separate. Draw off the lower layer into another separator and repeat the extraction of the liquid in the first separator twice, using 15 cc. of a mixture of one volume of alcohol and two volumes of chloroform, each time, and adding these extractions to the extractive in the second separator. Shake the combined extractions with 10 cc. of the acidulated water and allow the mixture to separate. Draw off the lower layer into a tared flask, and repeat the extraction of the acid liquid twice, using 15 cc. of fresh alcohol-chloroform mixture each time. Evaporate the combined chloroform extractions and dry the residue to constant weight at 100°C.

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[1] *J Ind Eng Chem*, 1914, 6 671

The results obtained by the U. S. P. IX method and by the Jenkins method on the Merrell specimen of podophyllum are respectively as follows:

	per cent
U. S. P. IX method	4.79 and 5.06
Jenkins method	6.04 and 6.05

It was noted that the resin obtained by the U. S. P. X method was not completely soluble in alcohol as is required by the U. S. P. X tests for resin of podophyllum, while the resins obtained by the other two methods were completely soluble in alcohol. It was also observed in the U. S. P. X assay that unless particular care was taken in filtering, some of the potassium citrate solution would escape through the filter with consequent augmentation of the results. The difficulty was overcome by decanting the alcohol-chloroform solution of the resin onto the filter after the aqueous layer had been drawn off as completely as possible.

From these few results it appears probable that the U. S. P. X method for the assay of podophyllum gives results that are above the truth. In order to test this supposition, two other specimens of *Podophyllum peltatum* were obtained, one from the Pharmacognosy Laboratory of the Bureau of Chemistry and the other from the Norwich Pharmacal Company. A specimen of Indian podophyllum (*Podophyllum emodi* Wallich) also was obtained from the School of Pharmacy of the University of Minnesota. Each of these was ground to a No. 60 powder, and each was assayed by each of the methods given in this paper. The results are tabulated herewith, the findings for Specimen I being included for comparison:

The results in Table 1 indicate still more pointedly the probability that the U. S. P. X method for the assay of podophyllum gives results that are considerably above the truth. However, in order to obtain further evidence several manufacturers who handle podophyllum were requested to assay a specimen of crude drug from their own stock by the U. S. P. IX method or by their own process of assay and by the U. S. P. X method. Only one report was received. The Norwich Pharmacal Company found 3.87 per cent of resin by its own method and 6.54 per cent by the U. S. P. X procedure. In reporting the results J. P. Snyder expressed the opinion that the U. S. P. method gave "entirely too high results". It should be noted that the higher result is about 70 per cent in excess of the lower.

TABLE 1.

Results of assays of podophyllum by several methods.

METHOD OF ASSAY	U. S. P. IX (ADAPTATION)	U. S. P X	JENKINS
	per cent	*per cent*	*per cent*
Podophyllum peltatum I. (Merrell)	4.79 5.06	6.99 6.83 7.14	6.04 6.05
Podophyllum peltatum II. (Bureau Specimen)	4.32 4.51	6.01 5.92	5.04 5.06
Podophyllum peltatum III. (Norwich)	4 90 4.86	7.02 7.00	6.33 6.42
Podophyllum emodi	17.09 17.08	19.77 20.04 19.93	17.65 17.68

A ground specimen of American podophyllum and one of Indian podophyllum were then sent to each of three schools of pharmacy with the request that they be assayed by each of the three methods. The results reported are not complete, but those returned are given in Table 2.

The results shown in Table 2 are conflicting, but in general they indicate that the U. S. P. X method gives higher results than the other two. Some of the findings by the U. S. P. X method, particularly in *Podophyllum emodi*, are so very high that it seems possible that the resin may have been contaminated with potassium citrate. The dangers from this source of error have already been mentioned.

TESTS FOR PURITY AND ACTIVITY.

Attempts were then made to determine whether the resin obtained by the U. S. P. X assay process was of the degree of purity required by the U. S. Pharmacopeia for resin of podophyllum and whether it was as active physiologically per unit of weight as the resin of the market. A tincture of podophyllum (2 liters), of such strength that 100 cc. of the preparation represented 10 grams of podophyllum, was prepared by percolation with hot alcohol. A quantity of resin was prepared from 200 cc. of this tincture by the U. S. P. X process. The yield was 7.19 per cent.

The U. S. Pharmacopeia requires that resin of podophyllum shall be completely soluble in alcohol, at least 65 per cent shall be soluble in chloroform, and at least 75 per cent soluble in ether. The resin was taken up in alcohol so far as possible, and to collect the alcohol-insoluble matter the solution was filtered through a weighed Gooch crucible. The residue was washed with alcohol, dried, and weighed. The alcohol-

TABLE 2.

Comparison of three methods for the assay of podophyllum by several investigators.

ANALYST	PODOPHYLLUM PELTATUM			PODOPHYLLUM EMODI		
	U.S.P.IX (Adaptation)	U.S.P.X	Jenkins	U.S.P.IX (Adaptation)	U.S.P.X	Jenkins
	per cent	*per cent*	*per een*	*per cent*	*per cent*	*per cent*
Marion G. Brimston	4.13	7.67		16.68	23.27	
College of Pharmacy	4.80	7.48		15.35	22.37	
University of Washington						
Violet Wong	4.95	6.29		13.00 13.82	44.47	
College of Pharmacy	3.12	5.67		13.74 13.73	35.37	
University of Washington						
F. J. Amrhein	8.43	9.13	7.72	11.25	28.62	18.46
Massachusetts College of Pharmacy						
T. R. Lund			7.76			18.39
Massachusetts College of Pharmacy						
R. S. Kelley	7.26	8.69	7.54	9.56	10.25	
Massachusetts College of Pharmacy						
M. J. MacLeod			5.84			
Massachusetts College of Pharmacy						18.78
H. J. Schaeffer	5.67			6.82		
Philadelphia College of Pharmacy and Science	6.22			6.54		
I. S. Mellonoff	5.90			6.70		
Philadelphia College of	5.61			6.91		
Pharmacy and Science	5.83					

insoluble material amounted to 0.37 per cent calculated to the original drug or to 5.1 per cent if calculated to the resin taken. The alcohol-soluble part was obtained by subtraction from 100 per cent. The proportions of the resin soluble both in ether and in chloroform were determined on other portions of the resin by the methods described in the U. S. Pharmacopeia, except that the methods were modified slightly by grinding the resin with sand in a mortar before extraction with the solvents. The findings are given in Table 3.

TABLE 3.

Solubility of resin of podophyllum (by U. S. P. X assay) in various solvents.

	ALCOHOL	ETHER	CHLOROFORM
	per cent	*per cent*	*per cent*
Found....	94.9	56.4	51.6
		56.4	53.4
U. S. P. requirement	100	75.0	65.0

Ash was also determined in a portion of the resin. The value found was 2.4 per cent. The U. S. Pharmacopeia permits not over 1.5 per cent of ash.

In order to test still further the quality of the resin obtained by the U. S. P. X assay it was subjected to the method recommended by Eder and Schneiter[1] for the determination of podophyllotoxin. For comparison two specimens of resin of podophyllum of known origin were also assayed by several collaborators and by the writer. The method is as follows:

ASSAY OF RESIN OF PODOPHYLLUM FOR PODOPHYLLOTOXIN.

Shake 0.5 gram of the finely powdered podophyllin in a stoppered flask with 15 cc. of chloroform frequently during half an hour. Filter through a dry filter, taking care to return the first few cc. of the filtrate to the flask and protecting the funnel and contents from evaporation as completely as possible by covering with a watch glass. Pour 10 cc. of the filtrate into 50 grams (80 cc.) of petroleum benzin contained in a tared Erlenmeyer flask. As soon as the precipitate has subsided, filter through a tared Gooch crucible and wash the precipitate and flask with 20 cc. of petroleum benzin. Dry the fractions of the precipitate in the Gooch crucible and in the flask for an hour at 70°C. and weigh[2]. The total residues, representing two-thirds of the podophyllin taken, should correspond to not less than 40 per cent of the weight of podophyllin taken.

The results are given in Table 4.

TABLE 4.

Assay of podophyllin for podophyllotoxin.

(Eder and Schneiter Method.)

ANALYST	SAMPLE NO 1	SAMPLE NO 2	SAMPLE NO. 3 RESIN BY U. S. P X ASSAY
	per cent	*per cent*	*per cent*
Brimston	52.53	44.25	
	53.46	44.58	
Wong	40.34	34.00	
	37.04	34.72	
Amrhein	53.68	51.60	
Kelley	55.87	51.88	
Warren	46.69	46.95	31.65
	45.85	47.22	33.89
	46.26		
	46.50		

The findings by the Eder and Schneiter method of assay for podophyllotoxin show that the resin obtained by the U. S. P. X assay process does not compare favorably with resin of podophyllum of the market.

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[1] *Pharm. Acta Helv.*, 1926, 1: 18.

[2] In the method as described by Eder and Schneiter it was directed that the residues of podophyllotoxin be left in the balance case for an hour before weighing. Miss Brimston observed that the podophyllotoxin was hygroscopic and that the weight should be taken directly from a desiccator This was confirmed by the observation of the writer.

The filtrate from the determination of the alcohol-insoluble part of the U. S. P. X resin was assayed for resin by the U. S. P. IX adaptation. The yield was 5.98 per cent as calculated to the original drug, or 83.2 per cent if calculated to the weight of resin taken. The acid filtrate remaining after this determination was subjected to the Jenkins method of assay. The yield was 0.18 per cent as calculated to the original drug or 2.5 per cent if calculated to the weight of resin taken. The sum of the alcohol-insoluble fraction, the U. S. P. IX fraction, and the Jenkins fraction is 90.8 per cent. On subtraction of this value from the yield obtained by the U. S. P. X process a loss of 9.2 per cent was found.

The alcohol-insoluble fraction was mixed with lactose, the mass was placed in capsules (11 mg. of extract), and the capsules were administered to cats by J. C. Munch and his assistants in the Pharmacological Laboratory of the Bureau of Chemistry. A trade specimen of resin of podophyllum was used as a control. The pharmacological tests indicated that the material was devoid of laxative properties. Other tests on a healthy man indicated that the material was inert.

The fraction obtained by the Jenkins assay process following the U. S. P. IX assay was subjected to pharmacological tests on man and cats. The results indicated that this fraction was about one-half as active as ordinary resin of podophyllum. However, since the percentage of this fraction present in the total resin is very small (2.5 per cent) this fact is not of great significance except to show that the U. S. P. method of preparing resin of podophyllum does not extract all the active constituents.

A slight excess of ammonia water was added to the residue in the separator after the Jenkins assay had been applied, the solution was evaporated almost to dryness, and the residue was mixed with lactose. The mass was then placed in capsules (each containing about 10 mg. of extract), and the activity was determined in the Pharmacological Laboratory. The results indicated that the residue was about one-half as active as U. S. P. resin of podophyllum.

The work was repeated with some modification. As carried out the second experiment was as follows: A quantity of the tincture (200 cc.) was assayed by the U. S. P. X method. The yield was 1.4156 grams or 7.08 per cent. This residue was taken up in alcohol so far as possible, and the alcohol-insoluble part was dissolved in water. The two solutions were mixed, the resultant solution was evaporated, and the assay was completed according to the U. S. P. IX method of assay. The yield was 1.0040 grams of resin or 5.10 per cent. The acid filtrate was then assayed by the Jenkins process for the assay of podophyllum. The resin obtained amounted to 0.0821 gram or 0.41 per cent. Pharmacological tests upon these several fractions gave results in substantial agreement with those obtained previously.

Some tests were made to ascertain whether the entire activity of podophyllum is obtained by the Jenkins method for the assay of the drug. Theoretically, two sources of error are possible in this assay, viz., (a) incomplete extraction by the alcohol in the first stage of the assay and (b) incomplete extraction by the alcohol-chloroform shaking-out process. It was demonstrated by appropriate tests that practically all the alcohol-soluble constituents are removed from the drug by the assay process, so that the error from the first source is negligible. In the Jenkins process the tincture of podophyllum is mixed with acidulated water and arbitrarily shaken three times with a mixture of chloroform and alcohol. It was found that when the shaking-out process was continued for several times the fourth, fifth, and sixth shake-outs yielded extractives so that the total yield of resin could be increased by several tenths per cent. The extractives obtained beyond the third shake-out were tested pharmacologically for laxative properties and were found to be inert. It was concluded, therefore, that all the medicinally active ingredients are removed by the Jenkins process of assay.

These tests indicate that resin of podophyllum obtained by the U. S. P. X assay contains a noticeable proportion of alcohol-insoluble material that is physiologically inert. Furthermore, the resin does not conform to the requirements of the U. S. Pharmacopeia in respect to ash or to solubility in ether or in chloroform. The experiments also show that the U. S. P. IX method for the assay of podophyllum (which is the same as the U. S. P. X process for the manufacture of resin from podophyllum) does not give all of the active ingredients in podophyllum. On the other hand, the Jenkins process for the assay of podophyllum apparently extracts all the active ingredients.

SUMMARY.

The U. S. P. X method for the assay of podophyllum gives results that are higher than those obtained either by the U. S. P. IX method or by the Jenkins process. The resin obtained by the U. S. P. X method of assay is not completely soluble in alcohol as is required by the U. S. P. X tests for the purity of resin of podophyllum, whereas the resin obtained either by the U. S. P. IX method or by the Jenkins process is completely soluble. Further, the solubility of the resin in ether and in chloroform is lower than the requirements of the U. S. Pharmacopeia. The ash limit of the Pharmacopeia is considerably exceeded. Pharmacologic tests on man and on cats demonstrate that the alcohol-insoluble portion of the resin (obtained by the U. S. P. X assay) is practically devoid of laxative properties. The resin, therefore, is not of U. S. P. quality. Quantitative tests indicate, also, that the resin obtained by the U. S. P. X assay process contains less podophyllotoxin than the resin on the market.

From the results of these studies it is concluded that the assay process directed by the U. S. Pharmacopeia for podophyllum gives results that are above the truth and produces a resin that contains inert material. This assay process is therefore unreliable. The Jenkins process is apparently satisfactory.

ACKNOWLEDGMENT.

The writer wishes to acknowledge his appreciation to the several pharmaceutical manufacturers and to others connected with schools of pharmacy who assisted in this study, either by contributing material or by collaborative work. The thanks of the writer are also due to the staff of the Pharmacological Laboratory of the Bureau of Chemistry for ascertaining the physiologic activity of the several podophyllum extractives.

JULIUS HORTVET, 1863—1927

JULIUS HORTVET

Who is the most enviable of men? He who has acquired wealth; or he who is greatly learned; or he who has gained wide control over the fortunes of others? Or is it he who—whether he be rich or learned or powerful, or the reverse of these—has won for himself and for what he has done the affection and the approval of his fellows? Judged by such a standard, Julius Hortvet must have been one of the happiest of men.

Of Scandinavian ancestry, he was born in Wisconsin in 1863. A country school near his home, and the Baraboo High School prepared him for entry into Wisconsin State University, whence he was graduated a Bachelor of Science in 1886. Moving to northwestern Minnesota, later to Minneapolis, he devoted many years to teaching in the public schools. During two years of this period he pursued a post-graduate course in chemistry at the University of Minnesota, but he did not continue it long enough to complete the work required for the doctorate. In 1900 he was appointed Chief Chemist of the Minnesota Dairy and Food Department, a position he held until his death. The biennial reports of the Department furnish ample proof of his industry and ingenuity.

His long—and most useful—service as a member of the Association of Official Agricultural Chemists began in 1900. A research dealing with the characteristics of maple sirup and with methods for detecting adulteration in maple-sugar products, published in 1904[1], was followed by his appointment as Associate Referee on Saccharine Products, including Confectionery. In 1905 he was made Associate Referee on Wine. In 1911 we find him Associate Referee on Beer; in 1912, Referee on Food Adulterations and Associate Referee on Dairy Products; in 1915, Referee on Dairy Products. This last-mentioned post he held up to the time of his death. Beginning in 1905, he presented nineteen reports to the association, dealing with Saccharine Products[2], with Colors[3], with Wines[4], with Food Adulteration[5], with Dairy Products[6], and with the Cryoscopic Examination of Milk[7]. It followed as a matter of course that he assisted in the work of compiling and editing the two editions of *Official and Tentative Methods of Analysis*.

During more than twenty years, therefore, he busied himself with the work of devising, testing, and improving methods of analytical procedure applicable to food products. Year after year he submitted reports of investigations, often of considerable length, and always characterized by thoroughness and impartiality. That he came to command a position of great influence would be only natural; yet, when his fellow-members sought, more than once, to honor him by his elevation to the presidency of the association, he would decline, pleading his deafness as an insuperable bar to his holding such a position.

Another kind of work for which he was admirably fitted was that of devising definitions and standards for food products. Consequently, from the time of his appointment, in 1912, until his death, he ably represented the association as a member of the Joint Committee on Definitions and Standards. It is betraying no secret to say that few, if any, of those who have held membership in that organization have contributed so much as he to the development of definitions and standards that would appeal to law administrators and to producers alike, by reason of their accuracy and of their fairness. Again and again has it happened, during sessions of the committee, that final decision would be postponed by vote of his associates until Hortvet was satisfied that every phase of the question had been adequately considered and that accurate analytical data, in sufficient volume, had been produced and studied. Should a hearing be under way, he would make every effort, with the aid of his acousticon, to follow the testimony brought before the committee: this required constant effort on his part, especially when a rapid fire of questions and answers was passing. At times he was evidently confused by his failure to catch the drift of the discussion: in his courteous way, he would ask that some statement be repeated for his benefit and, in a moment, would respond with a question or a comment that showed how quickly his mind sought out essentials and refused to be put off with trivialities.

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[1] *J. Am. Chem. Soc.*, 1904, 26: 1523-1545.

[2] Proceedings, 1905 meeting, 42-45.

[3] Proceedings, 1907 meeting, 9 (by title only).

[4] Proceedings, 1908 meeting, 12-25; 1909 meeting, 71-83.

[5] *This Journal*, 1915, 1: 110-113, 465-470.

[6] *Ibid.*, 1: 186-194, 538-544; 1916, 2: 238-257; 1920, 3: 436-446; 4: 201-210, 1921, 4: 482-491; 1923, 6: 422-429; 1924 ,8: 4-14; 1925, 8: 471-476; 1926, 9: 231-238.

[7] *Ibid.*, 1921, 4: 491-498; 5: 172-176; 1922, 5: 470-484 .

Thus, his fifteen years of service upon the Joint Committee were of great value. The oldest member in point of service, he was for years the spokesman for the Committee at the annual meetings of the Association of Official Agricultural Chemists. His were the only reports[1] upon the work of the committee which, in the opinion of the writer, ever gave an adequate impression of the amount and kind of labor that must be given to the task of formulating accurate food definitions and just food standards.

Although most of his written work was published in the Biennial Reports of the Minnesota State Dairy and Food Department and as referee's reports in the Proceedings of the A. O. A. C. and in *The Journal*, mention should also be made of his "Manual of Elementary Practical Physics"[2]; of his study of the acids of wines[3]; of his methods for the examination of maple products[4], of certain flavoring extracts[5], and for the determination of the oil of cloves[6]; and of an elaborate paper upon the cryoscopy of milk[7], in which appeal is made to chemists at large and not merely to those interested directly in food-law administration.

During the past ten years Hortvet devoted much patient study to the process of sublimation—especially when conducted under very low pressures—as a means of separating more volatile substances from admixture with less volatile impurities. He devised a form of "sublimator" with the aid of which he was able to separate from relatively non-volatile impurities such compounds as benzoic acid, salicylic acid, saccharin, caffein, etc., in quite pure condition, and to make quantitative determinations. By means of this apparatus he was enabled also to secure characteristic crystalline sublimates of substances contained in but minute quantity—*e. g.*, arsenic—and, under the microscope, to establish the fact of their presence[8].

His ingenuity showed itself in many other ways. He devised a form of graduated centrifuge tube for determining (from its volume) the amount of lead precipitate yielded by maple-sap products. He improved upon the types of distilling apparatus employed to determine the volatile acids of wines, etc. He designed a special form of Babcock bottle which enabled him to determine quickly the fat content of butter, and a caliper for the more accurate reading of Babcock milk bottles. His milk cryoscope enables the chemist to determine the freezing point of milk with such accuracy that the presence of as little as five per cent of added water can be established with certainty.

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[1] *This Journal*, 1923, **6**: 292-296; 1924, **7**. 292-293; 1925, **8**: 284-287; 1926, **9**: 103-107; 1927, **10**: 87-92.

[2] H. W. Wilson Co. (Two editions: 1900, 1902.)

[3] *J. Ind. Eng. Chem.*, 1909, **1**: 31-38.

[4] *U. S. Dept Agr., Bur. Chem.* Circ. 23.

[5] *J. Ind. Eng. Chem.*, 1909, **1**: 84-95 (in collaboration with R. M. West).

[6] *This Journal*, 1915, **1**: 154-157.

[7] *J. Ind. Eng. Chem.*, 1921, **13**: 198-208.

[8] *This Journal*, 1923, **6**: 481-489; 1925, **8**: 559-566.

In his work of research Hortvet was exact, thorough, ingenious. With all his thoroughness, however, he seemed to realize when absorption in detail failed to be longer of use and to see clearly the line that separated the really practicable from the merely possible. He was extremely industrious and painstaking. While the writer worked much with him, it was invariably away from his home, under conditions that called for haste; yet his day-to-day habit was manifest even then. That his scientific work, especially in connection with dairy products of all kinds, was of outstanding importance, is known the country over. What gave it special value in the eyes of all were his wide knowledge and his evident impartiality.

And now with regard to the man, as distinguished from the record of his scientific achievement. The writer cannot remember when he first met Hortvet: looking back over the period of their acquaintance—of their friendship—it seems to have existed always. Hortvet did not win friends through any conscious, apparent effort to draw men to him: he simply showed them, through his quiet talk and courtly ways, what manner of man he was, and so drew from them an equally sincere showing of what was in them. Hence, with him, other men were their true selves, were genuine; they could not pose nor affect a nature that was not really theirs. He led one to avoid exaggeration and to shun superlatives. Yet this influence was in no way based upon a capacity for caustic speech; that he may have had: if so, he did not use it. It was due to the simple frankness with which he expressed his thoughts, to the scorn he evidently felt for anything suggesting hypocrisy, and to the kindliness which never allowed him to hurt another's feelings, even in cases where the provocation must have been strong.

When all has been said, it was the character and the disposition of the man, rather than his demonstrated ability, that won for him the respect and affection of those who were privileged to know him. Integrity, geniality, humor, patience under a handicap such as renders many a man querulous or bitter—these qualities and gifts, native or developed, compel our admiration.

Death came to him at his home in Minneapolis on April 7 last. Surviving him are his widow, a son, three daughters, and a step-daughter. His body was interred in Lakewood Cemetery.

Vale, amice dulcissime!

WYATT W. RANDALL.

FIRST DAY.

MONDAY—AFTERNOON SESSION—Continued.

REPORT ON DAIRY PRODUCTS.

By JULIUS HORTVET[1] (State Dairy and Food Department, St. Paul, Minn.), *Referee.*

At the meeting held in October, 1925, the cryoscopic method was adopted as a tentative method for the determination of added water in cream, with the recommendation that it be submitted to collaborative study during the ensuing year, and that this study include tests applied to samples containing added water. In accordance with this recommendation the referee prepared the following outline of instructions to be submitted to collaborators:

1. *Samples.*

Five to ten samples of cream, together with corresponding samples of the milks from which the creams were derived, shall be used. The samples shall be authentic and shall be taken at different times and places. The cream samples shall be separated under the supervision of the analyst or the inspector who is entrusted with their collection. They shall test within a range of approximately 15 to 25 per cent fat. High-fat samples are undesirable owing to their high viscosity at low temperature, which makes it difficult, at times, to obtain a satisfactory freezing-point result. It will be most desirable to obtain results on samples testing (a) near the legal standards of 18 to 20 per cent fat, and (b) within the range of samples yielding low fat results, owing either to incomplete separation of cream or to dilution with water.

2. *Methods.*

Samples are to be subjected to tests before they have undergone any marked change in character. Determinations are to be made on the samples of cream and on the corresponding samples of milk. Acidity, total solids, non-fat solids, fat (Babcock method), and the freezing-point determinations are to be made on all samples, following the directions given in Chapter XIX of *Methods of Analysis*. Before applying the cryoscopic tests devote some time to preliminary trials on various kinds of samples, in order to insure ability to obtain good check results.

3. *Tabulation.*

Tabulate results that are well verified, discarding figures that are apparently erratic or not sustained by carefully made check determinations. Add to the tabulation any comments, criticisms, or suggestions that may have a bearing on the technique of the cryoscopic test in connection with its application to samples of cream.

4. *Watered Samples.*

Make cryoscopic tests on several samples of cream diluted with water. It will not be

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[1] Died April 7, 1927.

necessary to test more than a short series prepared from each sample. Dilutions covering a series from 10 to 20 per cent added water, by volume, ought to answer the purpose, viz., 10, 15, 20. Carry out cryoscopic tests on as many groups as can be conveniently handled. Include in the tabulation figures showing the actual amount of water added to each sample, the freezing point of the original sample, of each diluted sample, and the percentage of added water indicated by the freezing-point test.

Toward the close of the summer reports were received from the following collaborators: F. J. Doan, State College, Pa.; S. H. Hall, State Department of Public Health, Boston, Mass.; W. T. Mathis, Agricul-

TABLE 1.

Results obtained on undiluted samples.

SAMPLE NO.	WHOLE MILK				CREAM				SKIMMED MILK			
	Fat	S-N-F	Acidity	Freezing-Point Depression	Fat	S-N-F	Acidity	Freezing-Point Depression	Fat	S-N-F	Acidity	Freezing-Point Depression
	per cent	*per cent*	*per cent*	°C.	*per cent*	*per cent*	*per cent*	°C.	*per cent*	*per cent*	*per cent*	°C
F. J. Doan												
1	....	...	...	0.558	...	....	....	0.559	....	....	....	0.558
2	....	....	....	0.537	...	....	....	0.540	....	....	...	0.538
3	....	....	....	0.546	....	....	....	0.546	....	....	....	0.548
4	....	....	...	0.544	....	....	....	0.545	....	....	...	0.545
5	....	....	...	0.549	....	....	....	0.550	....	....	....	0.548
6	4.30	8.47	0.146	0.545	22.0	6.85	0.121	0.545	0.03	8.91	0.157	0.546
7	3.95	8.51	0.162	0.538	19.5	7.91	0.156	0.539	0.04	9.05	0.165	0.536
8	4.05	8.53	0.145	0.540	27.5	6.32	0.115	0.538	0.04	9.02	0.154	0.537
9	4.00	8.53	0.156	0.543	24.0	7.02	0.121	0.545	0.03	9.41	0.161	0.542
10	4.05	8.36	0.141	0.541	20.0	7.25	0.118	0.540	0.03	8.80	0.148	0.540
S. H. Hall												
1	3.00	8.23	0.141	0.537	18.70	7.03	0.116	0.537	....	...	....	....
2	4.05	8.48	0.160	0.547	18.60	7.08	0.140	0.549	....	....	...	....
3	4.20	8.72	0.137	0.548	19.70	6.59	0.127	0.550	....	....	....	....
4	3.55	8.56	0.135	0.539	18.90	7.12	0.122	0.542	....	....	....	....
5	3.35	8.07	0.135	0.539	15.80	7.24	0.124	0.543	....	....	....	....
6	4.20	8.51	0.133	0.540	18.80	7.54	0.111	0.541	....	....	...	....
7	3.50	8.53	0.136	0.535	19.00	7.31	0.120	0.535	....	....	....	....
8	3.40	8.02	0.162	0.548	20.10	6.62	0.140	0.549	....	....	....	....
9	4.20	8.55	0.146	0.547	19.30	6.67	0.123	0.548	....	....	....	....
10	3.60	8.26	0.116	0.533	18.60	8.05	0.098	0.532	....	....	....	....
11	3.70	8.28	0.123	0.545	24.10	5.94	0.099	0.548	....	....	....	....
W. T. Mathis												
1	....	....	....	....	24.0	5.24	0.13	0.540	....	....	....	....
2	....	....	....	....	23.3	5.53	0.21	0.562	....	....	....	....
R. W. Titus												
1	3.85	8.78	0.15	0.545	14.5	7.55	0.13	0.546	....	....	....	....
2	4.35	9.19	0.15	0.537	18.0	7.38	0.12	0.543	....	....	....	....
3	3.60	8.85	0.15	0.539	20.0	7.03	0.12	0.539	....	....	....	....
4	3.65	8.69	0.14	0.539	22.0	7.24	0.11	0.539	....	....	....	....
5	4.00	9.02	0.14	0.528	16.0	7.90	0.12	0.528	....	....	....	....
6	4.00	8.77	0.15	0.528	19.0	8.28	0.13	0.528	....	....	....	....
7	5.00	9.52	0.15	0.528	17.0	7.22	0.13	0.528	....	....	....	....
8	3.30	9.09	0.14	0.546	20.0	6.50	0.13	0.538	....	....	....	....

tural Experiment Station, New Haven, Conn.; and R. W. Titus, State Agricultural College, Manhattan, Kans. The reports of the collaborators are included in Tables 1 and 2 and in the comments following.

TABLE 2.

Results obtained on watered samples of cream.

SAMPLE NO.	FREEZING-POINT DEPRESSION, °C.						ADDED WATER CALCULATED BY FORMULA				
	Undiluted	Added Water—%					Added Water—%				
		5	10	15	20	25	5	10	15	20	25
F. J. Doan							$W = \frac{\text{\% Serum* in Cream } (T - T')}{T}$				
6	0.545	.	0.465	0.439	0.386	0.355	. .	11.0	14.6	22.0	26.3
7	0.539	. .	0.464	0.436	0.386	0.357	. .	10.7	14.8	22.0	26.1
8	0.538	.		.	0.384	. .	.	. .		20.0	.
9	0.545	.	0.461	0.417	0.386	0.363	.	11.3	17.2	21.3	24.5
10	0.540	.	0.465	0.414	0.379	0.353		10.6	17.9	23.0	26.7
S. H. Hall							$W = \frac{100 - \text{\% Fat } (T - T')}{T}$				
1	0.537	0.503	0.468	0.438	0.404	0.374	5.2	10.6	15.2	21.0	26.1
2	0.549	0.516	0.484	0.455	0.418	0.387	5.0	9.9	14.4	20.3	25.4
3	0.550	. .	. . .	. . .	. . .	0.383	. . .	.		.	25.9
4	0.542	0.508	0.476	0.441	0.413	0.382	5.1	10.1	15.6	20.2	25.3
5	0.543	.			.	0.386		.			25.5
6	0.541	0.502	0.466	0.439	0.407	0.377	5.9	11.5	15.8	21.0	26.0
7	0.535	0.505	0.482	0.441	0.402	0.384	4.6	8.2	14.7	21.0	24.2
8	0.549	0.516	0.476	0.444	0.410	0.385	4.9	10.9	15.9	21.2	25.4
9	0.548	0.510	0.475	0.442	0.409	0.383	5.7	11.0	16.2	21.5	25.8
10	0.532	0.499	0.463	0.430	0.400	0.371	5.1	10.8	16.1	21.1	26.1
11	0.548	0.511	0.471	0.430	0.398	0.368	5.2	11.0	17.1	22.1	26.9
W. T. Mathis							$W = \frac{\text{\% Serum* in Sample } (T - T')}{T}$				
1	0.540	0.503	0.466	. .	0.399	.	5.16	10.50		20.66	. . . .
2	0.562	0.524	0.487	.	0.422	. .	5.14	10.29	. .	19.79	. . .
R. W. Titus							$W = \frac{\text{\% Serum* in Cream } (T - T')}{T}$				
3	0.539	. .	0.467	0.435	0.402	. . .	. . .	10.3	14.9	19.6	. . .
4	0.539	. . .	0.466	0.425	0.414	0.374	. . .	10.2	15.9	17.4	23.0
5	0.528	. .	0.461	0.425	0.393	0.352	. . . .	10.2	15.8	20.7	27.0
6	0.528	. .	0.464	. . .	. .	.	. . .	9.4	. .	.	.
7	0.528	. . .	.	0.425	0.410	0.376	.	. . . .	15.6	17.9	23.1
8	0.539	.	0.466	0.446	0.404	0.384	. . .	10.2	12.9	18.8	21.6

* Per cent serum = 100 − [% fat + (0.38 × S-N-F)]. Results calculated on basis of *fat* and *S-N-F* in original samples.
T = freezing point of original sample.
T' = freezing point of diluted sample.

COMMENTS OF COLLABORATORS.

F. J. Doan.—Five authentic samples of herd milk (Nos. 6 to 10) were obtained from various sources, analyzed for acidity, fat, and solids-not-fat and their freezing-point depressions noted, after which they were separated and the same data obtained on the resulting cream and skim milk. Five other samples were studied with reference to the freezing-point depression only. The samples of milk were taken and separated within 12 hours of milking and in the interval were kept under 50°F. As soon as all the milk had been separated, samples were immediately taken in liter Erlenmeyer flasks and placed on ice, together with a similar sample of the original milk taken from the separator bowl. These samples were kept in cracked ice until analyzed, and for the freezing-point and acidity results this period was never over 4 hours. The greatest variation noted between the whole milk and its component cream and skim milk was 0.003°C., which is almost within experimental error. Variations noted were not consistently lower nor higher for the cream or skim-milk samples than for the samples of original milk. Variations in composition and acidity had no effect on this relation between the whole milk and the separated portions. From these observations it may be concluded that the freezing-point depressions of samples of cream and skim milk are identical with those of the whole milk from which they were derived; furthermore, that the depression of the freezing point depends entirely on the concentration of substances in the serum, neither the amount of fat present as an emulsion nor the amount of protein present as a colloid having any appreciable effect. The five samples of cream were diluted at the rate of 10, 15, 20, and 25 per cent with water, and additional freezing-point observations were made. The dilutions were made on a volume basis. Thus, for a 20 per cent dilution, 80 cc. of the cream sample was well mixed with 20 cc. of distilled water.

The amounts of added water were obtained by calculations made according to the formula given for milk. The results were uniformly high, as would be expected, since the added water affects the serum but not the bulk of the cream, so far as the freezing point is concerned, and the higher the fat content the lower will be the amount of serum. Consequently, a given amount of added water will lessen the freezing-point depression to a greater extent in samples of rich cream than in samples of thin cream. Carrying this idea to its ultimate conclusion, of course, it is seen that the formula is not theoretically correct even for milk, but the amount of substances other than serum present in milk is not large enough to affect greatly the use of the formula, especially where the average freezing point of milk is used for the value of *T*. The formula was corrected by relating the change in freezing point to the cream serum instead of to the bulk of the cream. This formula was as follows:

$$W = \frac{\%\ \text{Serum in Cream}\ (T - T')}{T}.$$

Here again the value of *T* was taken as the depression of the undiluted sample of cream. The percentage of serum was obtained from the analysis as follows:

$$\%\ \text{Serum} = 100 - (\%\ \text{fat} + \%\ \text{protein}).$$

Since the cream was not analyzed for protein, this figure was obtained by assuming that 38 per cent of the S–N–F consisted of protein.

Data were obtained on two samples of herd milk, after which one was diluted at the rate of 11.5 per cent with water and the other to the extent of 20 per cent. Data were also obtained on the diluted milk and again on the cream and skim milk obtained by separating the watered samples. The depression of the freezing point is essentially the same for the diluted milk and for the cream and skim milk derived therefrom, as shown in the following tabulation:

S. H. Hall.—Eleven samples of herd milk of known purity were obtained at different times and from different sources. In each case, after reserving a portion for analysis, the milk was separated, the skim milk being discarded. Determinations of fat, solids, acidity, and freezing point were made on the original sample of milk and on the cream. Portions of the cream were then diluted so as to contain, respectively, 5, 10, 15, 20, and

SAMPLES	FAT	S-N-F	ACIDITY	FREEZING-POINT DEPRESSION
	per cent	*per cent*	*per cent*	°*C*
Undiluted whole milk				
1	4.30	8.68	0.146	0.536
2	4.15	8.57	0.155	0.558
Diluted whole milk				
1—Diluted 11.5 per cent	3.80	7.76	0.135	0.462
2—Diluted 20 per cent	3.35	6.89	0.127	0.429
Cream from diluted whole milk				
1	25.00	5.97	0.106	0.461
2	21.05	5.92	0.085	0.430
Skim milk from diluted whole milk				
1	0.05	8.17	0.138	0.460
2	0.02	7.22	0.135	0.429

25 per cent added water. The dilutions were made by volume except in the cases of Nos. 5, 6 and 7, where they were made by weight. All the determinations were made in duplicate and each freezing-point result is the average of two determinations which checked within 0.002°.

The following formula for calculating added water is sufficiently accurate:

$$W = \frac{(100 - \% \text{ Fat})\ (T - T^1)}{T},$$

Where W = added water, T = freezing point of cream before dilution, and T^1 = freezing point of diluted cream. Since consideration of the fat alone will give calculated values so close to the actual values for added water, it seems unnecessary to complicate further the above formula in an attempt to correct for the colloidal protein. In the latter case, the fact should be considered that some of the protein, the albumin, is in true solution and consequently will depress the freezing point.

W. T. Mathis.—Samples in Series 1 are higher in acidity than in Series 2. This is because the tests and analyses were made the day following that on which the cream was separated. In Series 1 the analyses were begun immediately after separation. The increase in freezing-point depression due to increase in acidity follows the relation which we have found in our previous work, viz., 0.003°C. for each 0.01 per cent of acidity, except in the instance of 20 per cent dilution, but here the acidity value may be slightly too low.

R. W. Titus.—Eight authentic samples of herd milk, with the corresponding samples of cream, were obtained from the College Dairy Department. About 5 gallons of well mixed milk was emptied into a clean separator tank, and a quart sample was taken for analysis. The cream sample was obtained from the first third of the milk separated and was assumed to be a representative sample, since the bowl was clean previous to this operation. The samples were tested for fat, total solids, acidity, and freezing-point depression. The freezing-point depressions of the milks and the corresponding cream samples were almost identical, with two exceptions. The dilutions were made by adding, with a graduated pipet, the requisite amount of distilled water to a graduated test tube and then filling to the mark with cream. Six samples of cream were thus diluted, and the freezing-point determinations were made. From the data presented it appears that the freezing-point depression is not affected by the fat content of the sample, and no appreciable variation is shown between the freezing-point depression of the milk sample and the result on the corresponding cream sample.

DISCUSSION.

Results were reported on 31 samples of authentic whole cream and on 89 samples that were systematically watered. With the exception of a freezing-point depression of 0.528°C. on three samples reported by one of the collaborators, all the freezing-point results obtained on the unwatered samples occurred within the range reported in recent years as a result of collaborative work on authentic samples of milk. It is noted that these samples yielded fat results ranging from 14.5 to 27.5 per cent and acidity figures under 0.16 per cent.

The results reported on the watered samples are all apparently satisfactory, except in a very few instances. There appears to be no absolute criterion by which to judge these results unless, for the present at least, the formula adopted by Doan and Mathis is accepted. A casual consideration of the nature and composition of a sample of cream is sufficient to reveal the fact that the formula for calculating added water in milk is in need of modification. This matter is well stated in the comments submitted by Doan. As to the real merits of the two modes of calculation, the one proposed by Doan as compared with the one adopted by Hall, no definite statement can be made at present. It is apparent, however, that Hall's formula omits an essential factor, viz., the content of insoluble proteins. It has been impossible to verify the 0.38 per cent factor adopted in the calculation of the serum, but for the present this factor may be accepted as at least fairly representative of average cream. Doubtless the variations on either side of the figure are sufficient to account in part for the rather wide range shown in several instances between the calculated result and the known percentage of dilution. Further consideration of this matter may well be included in the work of the referee for the ensuing year. In spite of some discrepancies shown among results in Tables 1 and 2, two general conclusions may be stated:

First, the freezing-point tests applied to undiluted samples of normal cream are comparable with results obtained on samples of pure milk.

Second, as a means of determining quantitatively percentages of added water, the freezing-point test may be considered accurate to within a tolerance figure of 2 per cent.

An additional interesting feature shown among the results reported by collaborators is the fact that freezing-point results obtained on whole milk, on the cream separated therefrom, and on the skim-milk are practically identical. Also, freezing-point determinations applied by Doan to samples of watered milk indicated in each instance the same percentage of added water in the cream as in the original milk and in the skim-milk derived therefrom. In other words, separation of cream from watered milk does not disturb the composition of the serum. The soluble constituents in the serum remain substantially unchanged and consequently have the same effect on the freezing point.

OTHER METHODS.

The associate referee to whom was assigned a study of methods for the analysis of butter gave particular attention during the past year to methods for (a) sampling and (b) preparation of sample. It was impossible, owing to the comprehensive, thorough-going plan adopted for the collaborative work, to devote any attention to a study of methods for determining acidity and for distinguishing products made from pasteurized milk. The two remaining recommendations, (3) and (4), relating, respectively, to methods for the determination of albumin in milk and to a study of the suggestions made by H. C. Waterman[1] for the determination of casein, have not received consideration, chiefly on account of insufficient time and assistance available for a study of these subjects. The associate referee assigned to the subjects of malted milk and dried milk will present a report dealing with investigations that have been made during the past season. The Associate Referee on Ice Cream was appointed at a rather late date to fill a vacancy occasioned by the resignation of A. C. Dahlberg. However, a report embodying some substantial preliminary work will be presented at this meeting. A communication received from E. O. Huebner relative to the work on cheese constitutes essentially a report of progress and includes the following statement indicating the work accomplished during the past season:

> Experiments have been conducted on the normal calcium and phosphorus content of cheese, and it has been found (as one would expect) that the ratio of calcium to phosphorus is a rather constant factor. As disodium phosphate is used extensively as an emulsifier in process cheese, it will be seen that the use of this salt disturbs the normal calcium-phosphorus ratio and a high phosphorus content will be obtained. Thus, by determining the calcium and phosphorus in process cheese and knowing the normal ratio, one can calculate the added phosphate. A method has been applied to citric acid in cheese by which qualitative detection is certain, but quantitative results require further study. The method is an application to cheese of the oxidation of citric acid with nitric acid and the precipitation of the acetone dicarboxylic acid formed as its mercury salt. Although I have been unable to give any time except for reference work to the association this past year, I feel that some progress has been made and that in another year substantial progress can be reported.

A number of additional subjects have been brought to the attention of the referee. The first relates to the text of the brief instructions for determining specific gravity[2]. No criticisms have arisen relating to the determination of specific gravity by means of a pycnometer, but the alternative method referred to by the phrase "or by means of a standardized hydrometer", has been the subject of some correspondence. The referee's attention to this matter was directed by C. F. Hoyt, State Department of Agriculture, Sacramento, Calif., in connection with a special investigation that was carried out during the past year. A preliminary paper dealing with this subject has been prepared, and a well-rounded report will probably be published in the near future. After reading Hoyt's paper and conducting some correspondence, it was agreed that the directions for determining the specific gravity of milk by means of a hydrometer ought to be amplified. For the present purpose three points are suggested as desirable for consideration:

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[1] *This Journal*, 1926, 9: 246.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 259.

1. The description of the hydrometer,
2. The instructions for standardizing,
3. The instructions for reading.

Something should also be included relative to the construction of the cylinder—its height, diameter, and other essential features. It has been suggested that the hydrometer scale be changed so as to permit larger intervals. Much uncertainty relative to the correct method of reading the scale when the instrument is submerged in the fluid has prevailed for many years; that is to say, whether the reading should be at the top edge of the meniscus, somewhere near the surface of the liquid, or at a point somewhere intermediate. In order to arrive at a definite basis for a revision of the paragraph in question, it will be necessary to confer with the Bureau of Standards and with the instrument makers. It is evident that many individuals have no definite conception regarding the construction, graduation, and method of use of the milk hydrometer, and it is high time that attention be given to this matter, in order that the paragraph referred to may be improved.

QUALITATIVE METHODS.

A communication from W. D. Bigelow, Director of the Research Laboratories, National Canners Association, contains the following paragraph relative to the directions given for the testing of milk and milk products for the presence of gelatin[1]:

> We found that this method gave a positive test for gelatin with all the well-known brands of evaporated milk which we purchased on the Washington market. The precipitate obtained was of a flocculent nature that settled readily and which differed from that obtained with the same milk to which 0.1 per cent gelatin had been added. It was further found, however, that a negative test for gelatin was obtained when the evaporated milk was diluted one-to-one with water, in order to make it comparable in concentration with a fresh milk. However, a slight flocculent precipitate settled out on long standing (2 to 3 hours); a similar precipitate was obtained with fresh milk that had been heated either at the temperature of boiling water or at 240°F. Fresh milk itself gave a similar precipitate on standing overnight. From our recent experience it would seem advisable to insert in the method a paragraph on the preparation of the solution upon which the test is to be made. Of course, one would scarcely look for gelatin as an adulterant of sterilized evaporated milk, since gelatin is hydrolyzed at the temperature of boiling water and its thickening power destroyed. However, in the present case we may be somewhat embarrassed by the method as it is now

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 269.

given, as a foreign country is objecting to the importation of a shipment of American evaporated milk on the ground that it contains added gelatin.

Some correspondence was carried on relative to this subject, but no definite conclusions were reached. The last communication received from the laboratory of the National Canners Association is a repetition in part of the statements made by Bigelow in the earlier letter and represents the present stage of the investigations of the referee.

Early in the present year a questionnaire was prepared by Robert S. Breed of the Agricultural Experiment Station, Geneva, N. Y., and sent to laboratories engaged in the examination of milk. It was requested that the replies indicate any additional methods that seemed desirable to include in the standard methods of the American Public Health Association. Although the questionnaire dealt mainly with bacteriological subjects, some suggestions were brought out regarding chemical methods. A number of persons asked for the inclusion of a method by which they could detect the addition of hypochlorites; one person requested that a method be given by which the presence of neutralizer could be detected; another asked for a method by which it could be determined whether milk or cream had been frozen; another wanted a test for copper in milk; several wanted tests that would detect reconstructed milk and the presence of foreign fats; one desired tests for citric acid, particularly with reference to goat's milk; and several persons were interested in methods for making pH determinations.

It is evident from the foregoing that the scope of the study assigned to the various referees is increasing at a rate decidedly beyond their capacity to handle with the facilities now provided. None of the referees has been able during a given season to take care of more than one subject at a time owing to the burden of regular routine duties, and it is considered rather fortunate that so many of the referees have been able from year to year to prepare at least fairly acceptable reports. The circumstances indicated by the developments of the past year seem to demand at least two additional associate referees for the work on dairy products. Two suggestions are therefore presented:

First, that an associate referee be appointed on the subject of milk proteins with the recommendation that he devote his time chiefly to the suggestions given in the paper to be presented by H. C. Waterman. (This paper has been published[1].)

Second, that an associate referee be appointed with instructions to make a special investigation of the qualitative tests concerning which questions have been raised during the past year. Evidently these tests constitute a rather formidable list, but a judicious selection can be made so as to give attention to those that seem most important.

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[1] *This Journal*, 1927, **10**: 259.

RECOMMENDATIONS[1].

In addition it is recommended:

(1) That the cryoscopic method be adopted as an official method applicable to cream (second reading).

(2) That the methods for determining specific gravity of milk be given attention with a view to improvements in the directions.

(3) That the Associate Referee on Cheese be instructed to continue his study of methods for the detection and quantitative determination of emulsifying agents, preservatives, and other added substances.

(4) That the methods for the determination of albumin, including the suggested use of Almen's reagent for precipitating the albumin, be subjected to further consideration during the coming year; and also that the suggestions made by H. C. Waterman for the determination of casein in milk be a subject of study during the coming year.

(5) That tests applicable to the detection and determination of (a) gelatin, (b) neutralizers, (c) hypochlorites, (d) citric acid, (e) sucrose, (f) chlorine and chlorides, and (g) glycerin be studied by an associate referee.

REPORT ON BUTTER.

By LLOYD C. MITCHELL (U. S. Food and Drug Inspection Station, St. Louis, Mo.), *Associate Referee.*

The first report on butter made by this association was presented at the fourth annual convention in 1887, by H. W. Wiley[2]. On the four samples (oleomargarine, butterine, butter, and print butter) submitted for study, eight analysts reported the following maximum variations: for water, 1.46 to 3.15 per cent; for salt, 0.33 to 1.25 per cent; and for curd, 1.14 to 3.29 per cent.

The next and last report on butter, in so far as the determinations of moisture, non-fat solids, and fat are concerned, was made at the seventh annual convention in 1890, by E. H. Jenkins[3]. Eleven analysts reported the following maximum variations on one sample of creamery butter: water, 0.38 per cent; casein, 1.07 per cent; ash (salt), 0.53 per cent; and fat, 1.88 per cent.

The methods of analysis used in the above work are essentially the same as the official methods published in 1925[4]. The method of preparation of the analyst's sample adopted in 1890 is the same as the method published in 1920[5] except that the 1920 method gives instructions to cool the sample while shaking until it is solidified sufficiently to prevent the separation of the water and the fat. This addition was first inserted into the method by the Committee on Revision of Methods and adopted by the association in 1907[1]. In its latest edition of methods, 1925, the Committee on Editing Methods of Analysis further revised the method for the preparation of the sample by inserting the word "mix", deleting the instructions for cooling, and substituting the word "soften" for the word "melt". No collaborative study, however, has ever been made of any method for the preparation of the sample.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 71.
[2] U. S. Dept. Agr. Div. Chem. Bull. 16, p. 14.
[3] U. S. Dept. Agr. Div. Chem. Bull. 28, p. 48.
[4] *Methods of Analysis, A. O. A. C.*, 1925, 275.
[5] *Ibid.*, 1920, 232.

At the 1925 meeting of the association, an associate referee was appointed and directed to study the methods for the examination of butter, particularly methods for (a) sampling, (b) preparation of sample, (c) acidity, and (d) distinguishing the product made from pasteurized cream.

As the field for investigation was so wide and the available data were limited, the associate referee was unable to devote the necessary time to cover the assignment in a single year. A study of sampling of tub butter, in connection with other work, and an extensive collaborative study of the official method and a proposed method for the preparation of sample were made.

The sampling and the preparation of the analyst's sample, in particular, present peculiar difficulties. The different constituents, insoluble in one another, with the exception of salt in water (up to the saturation point), are unevenly distributed throughout the mass of butter, making it impossible to obtain a representative sample of bulk butter by the present methods. Even were the constituents uniformly distributed in the mass of butter, it would be rather doubtful whether a truly representative sample of bulk butter could be obtained by means of the present trier.

In the usual preparation of the sample, a certain portion of the constituents becomes separated, and must be reincorporated before the sample is ready for analysis. In practically every operation from the time the butter leaves the churn until the small portion is weighed for analysis the butter loses some moisture. This loss is probably most noticeable in the preparation of the sample and in the analysis of the butter.

The St. Louis Station[2] of the Bureau of Chemistry made an extensive study of the wedge method, the heated and cold trier methods, and the auger method for the sampling of tub butter. The conclusions drawn from the work follow:

(1) The wedge method is impracticable for sampling tub butter.

(2) The heated trier method for frozen butter and the cold trier method for butter above freezing invariably gave samples of butter containing lower moisture values, hence higher fat values, than the true moisture

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[1] U. S. Dept. Agr. Bur. Chem. Bull. 107, p. 123.

[2] Unpublished report, April, 1926.

content of the entire tub of butter, even when all the water or brine adhering to the trier was carefully transferred to the sample container.

(3) The auger method, applicable only to frozen butter, gave samples whose moisture content is believed to be nearer the true value than the samples taken by the trier methods.

(4) The variations in moisture content in different parts of any tub are likely to be so wide that to insure a representative sample it is necessary to draw many portions from all parts of the tub. This is obviously impracticable.

Owing to the non-homogeneity of tub or bulk butter, the complex conditions encountered in sampling, the difficulties inherent in the technique of transferring, heating, and emulsifying the entire-tub samples of butter (which is apparently the only basis for comparison), the time, effort, and expense involved in the sampling of many tubs of butter, and the complete mixing of each tub sampled, it is the opinion of the associate referee that collaborative study of the sampling of tub or bulk butter at the present time is practically impossible.

RECOMMENDATIONS FOR SAMPLING BUTTER.

Profiting by the results of the study made at the St. Louis Station and from experience, the following recommendations are offered tentatively:

(a) *Print Butter.*

(1) That each analyst's sample consist of the entire contents from one unit package or carton in case of pound or half-pound packages, or two unit packages or cartons in case of quarter-pound packages. The package or carton considered here refers to the product usually wrapped in a cardboard carton and not to any further subdivisions wrapped in parchment paper, the subdivisions being considered as part of the package or carton.

(b) *Tub or Bulk Butter.*

(1) That the trier, either cold or heated, be the instrument used to take the sample.

(2) That when the butter is packed in tubs, the trierful (core) be taken by inserting the trier vertically into and through the butter at a point two-thirds of the distance from the center to the edge of the tub.

(3) That any water or brine adhering to any part of the trier be carefully transferred to the container.

(4) That an inch of butter from the top of the core be replaced into the hole made by the trier.

(5) That each analyst's sample consist of three trierfuls (full cores) of butter. These may be from one tub or from each of three tubs from the same churning.

PREPARATION OF THE ANALYST'S SAMPLE.

The collaborative work undertaken this year on the preparation of butter samples for analysis was a comparison of the present official method and a proposed mechanical stirrer method[1]. The proposed method as submitted to the collaborators reads as follows:

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[1] *This Journal*, 1925, 8. 574.

Soften the sample, 250–500 grams, in a closed vessel to such an extent that on stirring for 2–3 minutes the product will reach a temperature of 31–34°C. Stir with a malted milk mixer for 2–3 minutes, with an up-and-down movement of the stirring device, at the same time slowly moving the vessel horizontally so that the stirrer reaches all parts of the sample. The final temperature must be 31–34°C. If the temperature is below 31°C., continue stirring until this temperature is reached. A temperature above 34°C. will indicate that the sample has been warmed too much and will probably separate. In this case, cool the sample until solid and repeat the warming and mixing. Weigh the portion for analysis within 3–4 hours with a room temperature of 25°C. or below, and within one-half hour with a room temperature of 28°C. or above.

In order to cover both tub or bulk butter and print butter at the same time, the collaborators were requested to prepare both pound and half-pound samples by each method. Three portions were taken from the upper surface of the prepared sample for analysis, one from the left side, one from the middle, and one from the right side. Then approximately two-thirds of the butter was removed, and three more portions from the new and lower surface were taken for analysis, one from the front, one from the middle, and one from the back. Moisture and non-fat solids were determined on each portion by the official methods. The fat was obtained by subtracting the sum of the results for moisture and non-fat solids from 100.00.

Unusual interest was shown in the work as evidenced by the number of extensive reports received by the associate referee. Several of the collaborators submitted other interesting items pertaining to the butter work which will be covered in the discussion.

No attempt is made to report each individual result submitted by the collaborators. To do so would lead to more or less confusion without bringing out the salient points of the two methods, namely, the homogeneity or non-homogeneity of the samples of butter as prepared by the methods studied. Table 1, therefore, was prepared to show the maximum variation obtained for each constituent determined in the different samples prepared and analyzed by the collaborators.

The results of this study were contributed by the following collaborators, to whom the associate referee at this time desires to express his thanks and appreciation:

L. T. Anderegg, Iowa State College, Ames, Iowa.

E. H. Berry and C. A. Roach, U. S. Food and Drug Inspection Station, Chicago, Ill.

F. W. Bouska, Beatrice Creamery Company, Chicago, Ill.

H. B. Ellenberger, Agricultural Experiment Station, Burlington, Vt. (J. A. Newlander, analyst).

L. D. Elliott[1], U. S. Food and Drug Inspection Station, Seattle, Wash.

Leonard Feldstein, U. S. Food and Drug Inspection Station, Denver, Colo.

L. W. Ferris, U. S. Food and Drug Inspection Station, Buffalo, N. Y.

H. W. Gregory, Purdue University, Lafayette, Ind. (George Spitzer and W. F. Epple, analysts).

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[1] Present address: U. S. Food and Drug Inspection Station, Denver, Colo

C. A. Greenleaf, U. S. Food and Drug Inspection Station, Cincinnati, Ohio.
E. S. Guthrie, Cornell University, Ithaca, N. Y.
R. L. Horst, U. S. Food and Drug Inspection Station, New Orleans, La.
J. T. Keister, Bureau of Chemistry, Washington, D. C.
T. O. Kellems, U. S. Food and Drug Inspection Station, San Francisco, Calif.
W. D. Richardson, Swift & Company, Chicago, Ill.
A. E. Rowe, U. S. Food and Drug Inspection Station, Philadelphia, Pa.
L. A. Salinger, U. S. Food and Drug Inspection Station, Savannah, Ga.
E. C. Scott, Michigan State College, East Lansing, Mich.
M. M. Simpson, The Fairmont Creamery Company, Omaha, Nebr.
H. R. Smith, U. S. Food and Drug Inspection Station, Baltimore, Md.

SUMMARY OF ANALYST'S COMMENTS.

Anderegg.—No marked difference in homogeneity was observed depending upon whether the sample was prepared at 27°C. or at 34°C., or whether the sample was agitated by shaking by hand or by the use of a mechanical stirrer. It is believed that a mechanical stirrer will save considerable time when many samples are to be prepared for analysis.

Berry and Roach.—We can see little, if any, advantage in the proposed method over the official method. It would seem that the emulsified condition in which the proposed method leaves the butter has some advantage. The present details, which call for so manipulating the operation that the stirrer reaches all parts of the sample, seem largely to have eliminated the difficulty of segregation.

Bouska.—I believe the principle of preparing a sample with a drink mixer is correct. Without further experience, however, I am compelled to give my preference to the official method.

Ellenberger.—The proposed method of preparing butter samples gives a homogeneous mass which checks closely, though no more closely than the official method, but the proposed method has a decided disadvantage in that considerable moisture (an average of 0.19 per cent in twenty tests) is lost in the process. Consequently, we cannot recommend the method. The loss is greater than the difference between check tests by either method. Variations due to conditions or methods of sampling are of much greater importance than the differences in check tests. In every trial except one the jars tested first by the official method gave higher results than jars tested first by the proposed method, the average difference in the eight comparisons being 0.19 per cent.

Elliott.—The proposed stirrer method gives slightly more uniform results throughout the jar, both in one-pound and half-pound samples, than the shaking method. However, if all the results are like mine, I would say there is very little to choose between the two procedures so far as the analytical results are concerned, and I would advocate making the procedure optional between the two methods, depending upon conditions. (Elliott also mentions the greater ease and quickness of the proposed method.)

Feldstein.—The official method was first favored because it was thought that the sample could be mixed better. I had noticed previously that with the malted milk stirrer there seemed to be a slight separation of fat on the surface, although good results were obtained. It was just this appearance which prejudiced me at first, but this slight apparent separation is so easily overcome by moving the jar and raising and lowering the stirrer, and by stirring a little longer, that now I prefer the proposed method.

Ferris.—The results obtained indicate that both the official method and the stirrer method as interpreted in this work give a sample that is satisfactorily uniform throughout when the sample consists of either one pound or one-half pound of butter.

Gregory.—The differences between the extremes of the determinations made from samples taken from the side, center, and opposite side of each lot of butter are no greater than variation between duplicate moisture and non-fat solids tests encountered constantly by the careful and skilled chemist. The moisture and non-fat solids are uniformly distributed throughout the entire sample when the sample is properly prepared by either the official or the proposed method. Butter prepared by the proposed method will remain homogeneous for at least 3 hours when held at a temperature of 24 to 25°C. Moisture determinations made from samples prepared by the proposed method are not entirely consistent with the moisture determinations made from samples prepared by the official method.

Horst.—It appears that the official method gives slightly higher average moisture content. We have been using the proposed method, supplementing it with thorough stirring of the sample with a spoon after mixing as much as possible with the electric stirrer.

Keister.—As to the merits of the two methods of preparing butter samples, I am frank to say that so far as my limited experience with the stirrer method goes, I see no greater accuracy by its use, and taking into consideration the extra equipment and somewhat greater time and effort, I would hesitate at the present time to favor its adoption; however, with more experience and results I may see an advantage in certain cases. The texture and other conditions of the butter no doubt play a part in determining the best treatment for moisture determinations.

Kellems.—Two difficulties were found in using the malted milk stirrer. First, the sample was not completely stirred. Some butter at the top around the edge was not mixed in with the stirred portion, and at the bottom of the jar, around the edge, there was even more unmixed butter than at the top. Second, the butter splashed up on the side of the jar and made the sample look rather sloppy. The malted milk stirrer does not mix the butter completely and is a little sloppy. Also more care is necessary in heating the butter to just the right temperature than is required in spatula stirring. Neither method is fool proof, but the spatula method, in my opinion, is more nearly so, and is easier of operation. In the main the results by spatula stirring are more uniform than those by the malted milk stirrer.

Richardson.—We have never encountered any difficulty in the preparation of butter samples for analysis. With the exercise of ordinary precautions, no trouble has ever been experienced in getting a homogeneous mixture of the product. The only advantage we see in the proposed method is the standardizing of certain of the details of the official method which are at present somewhat vague. Our results do not convince us that the temperature of mixing is vital in obtaining a uniform sample providing, of course, it is maintained high enough so that a homogeneous mixture may be secured by proper mixing.

Rowe.—In all cases the variation in moisture was less than the variation in non-fat solids. The proposed stirrer method is more accurate than the official method; the sample should be large enough to stir readily; and the sample should be thoroughly stirred by hand with a spoon immediately before weighing.

Scott.—Although the results of the analyses by both methods show several discrepancies, I believe that it would be possible to get results which check much more consistently with the mechanical mixer than by the old method—that of stirring by a rod or shaking the butter in the container. There is so much agitation by the mechanical mixer that, with very little care, a perfect emulsion of the water and non-fat solids in the fat should be obtained.

Simpson.—Samples prepared by either method are thoroughly uniform as shown by the small variation of the tests. The moisture tests are lowered by stirring with the malted milk stirrer. The stirrer method is time saving and less laborious. The results are uniform, although they are somewhat lower than those obtained by the official method and are lowered by increasing the period of stirring.

Smith.—In the second set of variations are included portions of butter scraped from the sides of the jar above the level of the mass of butter. Previous work had indicated that such samples would show less moisture and more fat than the main portion of butter. Since the results of these determinations are obviously in error, all of them were discarded in the first set of variations. This variation is not noticeable in the samples prepared by the stirrer method. A possible explanation is that by the official method the containing vessel is cooler than the sample and there would be a tendency for butter fat to congeal on the inside of the container during the first period of the shaking. In the stirrer method the container is warmer than the sample when stirring is commenced and furthermore, the stirrer, when properly manipulated, agitates the mixture on the sides of the vessel as well as the mass of sample. Of the two methods for preparing butter samples, the stirrer method is preferred for the following reasons:

More than one sample may be analyzed at a time. With the official method the personal attention of the analyst must be concentrated on one sample. The stirrer method permits an analyst to prepare a series of samples, keep them in the refrigerator until ready to weigh out, and then do all the weighing at one sitting.

If one determination has to be repeated the sample does not have to be reprepared. In one instance, a sample prepared by the stirrer method was checked after standing in the storeroom at room temperature for three weeks, and excellent duplicate determinations were obtained.

The stirrer forms an emulsion of water, curd, fat, etc., similar to a mayonnaise dressing. The water is distributed throughout the mass in finer globules than is possible with shaking by hand. The sample is proportionately more homogeneous and stable.

A large part of the personal equation in the preparation of the sample may be eliminated. The official method depends largely on a quick weighing out after the shaking. No two analysts will do this exactly alike.

DISCUSSION.

Nineteen collaborators prepared or reprepared one hundred and sixty-one samples of butter, and made eight hundred and forty-one moisture and six hundred and twenty-two non-fat solids determinations, from which six hundred and seventeen fat (by difference) values were calculated.

In following the official method, eight of the collaborators prepared the samples by softening or melting the butter in various ways and shaking vigorously until the butter mass became too stiff to shake, six cooling the butter by shaking in the air, one by allowing cold tap water to flow over the container, and one by setting the butter in water at 10°C. Eight of the collaborators softened the sample somewhat in various ways, then beat it to a creamy consistency by means of spatula, table knife, glass rod, spoon, or other device. One collaborator used a combination of the variations mentioned. The butter was softened and shaken, then the lid was removed, and the butter adhering to the lid and sides of the container was scraped off and added to the butter mass, which was then beaten to a creamy consistency by means of a spatula. Two did not report the method used.

It is apparent from these reports and comments that the official method is not a specific one, but allows a wide variation in technique. Whether or not this is desirable is questionable.

Two or three of the collaborators, who did not have a malted milk stirrer available, devised mechanical stirrers similar to the milk stirrer, and this substitution appeared to work satisfactorily.

Seven analysts indicated a preference for the proposed method, five for the present official method, four were undecided, and three indicated no preference. Four of those in favor of the proposed method and two in favor of the official method had had no previous experience with the mechanical stirrer in the preparation of butter samples, but on the other hand they had extended experience in the preparation of butter samples by some variation of the official method.

The results indicate that samples consisting of either a pound or a half pound of butter can be prepared satisfactorily. This fact simplifies the sampling of print butter in that the entire contents of a unit package can be used as an analyst's sample.

Of the 113 samples reported in Table 1, 72 per cent showed less than 0.20 per cent total variation in the moisture content, the maximum variation being 0.55 per cent and the average variation ranging from 0.13 to 0.18 per cent for half-pound and pound samples, as prepared by the two methods. Of the 93 samples for which the non-fat solids are given, 70 per cent showed less than 0.20 per cent in the total variation, the maximum variation being 0.77 per cent and the average variation ranging from 0.15 to 0.21 per cent. Of the 93 samples for which the fat (by difference) was calculated, only 45 per cent showed less than 0.20 per cent in the total variation; 61 per cent showed 0.25 per cent or under in the total variation, the maximum variation being 0.92 per cent and the average variation ranging from 0.22 to 0.31 per cent for half-pound and pound samples prepared by the two methods.

The amount of non-fat solids in salted butter ordinarily is about 20 to 25 per cent of the water content, although the results in Table 1 show a greater variation in the non-fat solids content than in the moisture content. It is a question whether this discrepancy is due to lack of homogeneity of the sample or to errors in the determination of the non-fat solids. It is noted, however, that as much as 1 gram of asbestos is contained in the Gooch crucibles used by some of the collaborators for the determination of the non-fat solids[1]. There is reason to believe that this may involve a significant error, due to retention of fat by the asbestos. Other errors that may be involved in the official method undoubtedly play a part in some of the variations obtained by the collaborators.

Feldstein called attention to the fact that the malted milk stirrer was designed to mix substances in a vessel not over 2½ inches in diameter, while the Mason jar used as a container suitable for butter samples had a diameter 3 to 3½ inches. With a view to obviating the necessity of moving the jar, he suggested slightly increasing the size of the stirring disc, thus decreasing the liability of breaking the container. From certain considerations, which will not be discussed at this time, it is doubtful whether this modification would prove useful owing to the decreased mixing action in a vertical plane.

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[1] *This Journal*, 1926, 9: 209.

Two or three of the collaborators suggested tilting the jar during the stirring so that a stream of agitated butter hits the bottom and sides of the jar at an angle. This is in addition to the horizontal movement of the jar and the up-and-down movement of the stirring device. Tilting the container in this manner gives a stronger whirling action and is particularly effective in incorporating the butter at the bottom and on the sides of the jar with the remainder, but it increases the risk of breaking the container if it is made of glass.

Kellems[1] submitted some interesting comparative data on a direct determination of fat in butter. Using a modification of the Schmidt-Bondzynski method for the determination of fat in cheese, he obtained variations of 0.02, 0.03, 0.12, 0.17, 0.14, 0.11, 0.41, and 0.18 per cent, an average difference of 0.03 per cent higher results than those obtained by the present official method. This adaptation appears to present for collaborative study a method for the direct determination of butter fat, the most important constituent of butter.

Ellenberger, Gregory, and Simpson observed that the moisture tests are lower on those samples prepared by the proposed method that are first prepared by the official method. Both Ellenberger and Gregory prepared their samples by the official method by softening in closed containers and shaking, though the details of their procedures differ. Simpson softened the butter, spread it over the entire inner surface by shaking, and then removed the lid and scraped off the butter adhering to the lid and sides of the container with a spatula, adding this to the butter mass and finally beating up the whole mass to a creamy consistency by means of a spatula. Allowing for the moisture loss during the operation of removing two-thirds of the butter to take the lower layer of samples and returning this butter to the jar, the discrepancy observed by Ellenberger between the moisture results obtained by the official and proposed methods amounts to approximately 0.10 per cent. This difference was caused by the evaporation of moisture during the preparation of the sample by the proposed method.

Gregory found an average loss of 0.10 per cent moisture on 12 samples, first prepared by the official method and sampled (including removal of two-thirds of the butter) and then recombined and prepared by the proposed method. The actual error due to loss of moisture during the preparation of the proposed method was therefore less than 0.10 per cent and, to judge by Ellenberger's results, probably not over 0.05 per cent.

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[1] Work suggested by H. J. Wichmann, U. S. Food and Drug Inspection Station, San Francisco, Calif.

Simpson found a discrepancy of 0.25 and 0.14 per cent, respectively, on two samples. He repeated the work on four other samples, 100 grams in size, without removing two-thirds of the butter from the jar and recombining, and found an average loss of 0.09 per cent of moisture.

From these results, it seems clear that from 0.05 to 0.10 per cent of moisture is lost during the preparation of the sample by the proposed method. The work in the associate referee's laboratory, where the proposed method is a familiar one, has disclosed a usual loss of 0.05 per cent in repreparing by the proposed method samples that had been prepared by the same method. Before any definite conclusions are drawn on this point, it will be advisable to conduct experiments in which samples will be (1) first prepared by the official method and then reprepared by the official method, both the spatula and shaking modifications being used, (2) prepared by the proposed method and then reprepared by the proposed method, (3) prepared by the proposed method and then reprepared by the official method (both modifications), and (4) prepared by the official method (both modifications) and reprepared by the proposed method. It would be highly desirable to use synthetic butters of known composition, provided mixtures could be obtained that had the same consistency and physical structure as natural butter, but so far this has been practically impossible.

The report of Ellenberger brings up another point. Seven of the eight sets of samples, each set consisting of two samples taken from the same tub or churning, showed somewhat higher moisture when first prepared by the official method than when first prepared by the proposed method. This difference might possibly be caused by the condensation of moisture, either originally in the air in the container or evaporated from the butter into the air-space, on the lid and sides of the container, this condensate being incorporated into the samples as prepared by Ellenberger according to the official method, but not being incorporated into the samples prepared according to the proposed method.

SUMMARY AND RECOMMENDATIONS.

Of the nineteen collaborators engaged in this work, seven preferred the proposed mechanical stirrer method, five preferred the present official method, and the remainder were either undecided or indicated no preference.

Taking into consideration that the proposed method was used for the first time by collaborators who were unfamiliar with the manipulative details, the results obtained by this method are distinctly encouraging, and on the whole they are more promising than those obtained by the official method.

TABLE

Maximum

COLLABORATOR	OFFICIAL METHOD							
	½ pound in pint container				1 pound in quart container			
	Moisture	Non-fat solids	Fat	Final temperature at which mixed	Moisture	Non-fat solids	Fat	Final temperature at which mixed
	per cent	*per cent*	*per cent*	°*C.*	*per cent*	*per cent*	*per cent*	°*C.*
1	0.05	0.02	0.06	26	0.04	0.10	0.12	26
	0.04	0.05	0.06	31	0.10	0.04	0.10	31
					0.05	0.07	0.03	34
					0.06	0.04	0.07	30
2	0.23	0.15	0.26	24	0.25	0.23	0.38	24
3	0.36	0.15	0.43	25	0.23	0.20	0.20	25
4	0.09			20–22	0.08			20–22
	0.07			20–22	0.11			20–22
	0.05			20–22	0.06			20–22
	0.15			20–22	0.07			20–22
5	0.14	0.06	0.17		0.11	0.16	0.20	
6	0.17	0.20	0.31	26.4	0.30	0.14	0.33	28.2
7	0.09	0.18	0.21	28.5	0.06	0.15	0.15	30
8	0.13	0.26	0.31	33–35				
	0.17	0.12	0.22	33–35				
	0.26	0.08	0.33	33–35				
	0.15	0.19	0.25	33–35				
	0.24	0.08	0.31	33–35				
	0.47	0.06	0.46	33–35				
	0.11	0.32	0.38	33–35				
	0.20	0.13	0.13	33–35				
	0.14	0.13	0.16	33–35				
	0.15	0.17	0.21	33–35				
	0.26	0.13	0.19	33–35				
	0.17	0.22	0.29	33–35				
9	0.02	0.19	0.17	31				
10	0.11	0.35	0.21		0.20	0.09	0.33	
	0.16	0.64	0.64		0.16	0.34	0.57	
11	0.26	0.25	0.42		0.17	0.11	0.22	
12	0.10			28	0.19			28.5
13	0.08	0.08	0.14	27–29	0.06	0.11	0.11	27–29
14	0.05	0.10	0.15	26	0.08	0.11	0.17	24
15*	0.11	0.22	0.24	28	0.25	0.22	0.19	28
16	0.11	0.13	0.17		0.12	0.27	0.22	
17	0.15	0.25	0.16	32	0.19	0.28	0.44	32
18	0.05	0.10	0.11	25	0.10	0.08	0.14	27
19	0.10	0.03	0.12	30	0.09	0.14	0.13	30
†	0.50	0.12	0.58		0.25	0.14	0.27	
Maximum	0.47	0.64	0.64	33–35	0.30	0.34	0.57	32
Minimum	0.02	0.02	0.06	20–22	0.04	0.04	0.03	20–22
Average	0.15	0.17	0.24	....	0.13	0.15	0.22	....

* Used 150 and 300 grams, respectively, instead of ½ and 1 pound samples.

† Actual variations obtained. See comments for explanations. Not included in maximum, minimum, or average.

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variations.

PROPOSED METHOD

½ pound in pint container				1 pound in quart container			
Moisture	Non-fat solids	Fat	Final temperature at which mixed	Moisture	Non-fat solids	Fat	Final temperature at which mixed
per cent	*per cent*	*per cent*	*°C.*	*per cent*	*per cent*	*per cent*	*°C.*
0.07	0.07	0.12	27	0.08	0.05	0.12	34
0.23	0.25	0.35	33	0.28	0.30	0.43	34
0.16	0.27	0.40	31–34				
0.06			31–34	0.02			31–34
0.17			31–34	0.08			31–34
0.21			31–34	0.13			31–34
0.10			31–34	0.06			31–34
0.15	0.10	0.19	32	0.14	0.09	0.18	32
0.22	0.25	0.22	32.4	0.33	0.16	0.30	33
0.25	0.10	0.29	32.5	0.13	0.11	0.14	32.5
0.09	0.11	0.20	34.0				
0.15	0.18	0.30	34.3				
0.24	0.15	0.32	33.0				
0.13	0.12	0.22	32.9				
0.18	0.08	0.26	33.1				
0.20	0.08	0.25	34.2				
0.39	0.31	0.66	34.0				
0.37	0.13	0.37	34.0				
0.20	0.22	0.41	33.5				
0.09	0.11	0.16	33.0				
0.21	0.16	0.23	34.2				
0.55	0.14	0.54	34.0				
0.10	0.43	0.52	33				
0.10	0.77	0.87		0.14	0.54	0.58	
0.27	0.75	0.92		0.22	0.65	0.61	
0.24	0.30	0.22	33	0.10	0.14	0.14	31.5
0.31			31	0.13			31.0
0.12	0.11	0.16	31–33	0.45	0.09	0.47	31–33
0.05	0.10	0.12	31.7	0.12	0.11	0.11	32.8
0.13	0.21	0.13	32.8	0.05	0.12	0.13	33.2
0.04	0.07	0.11	31–33	0.06	0.08	0.11	31–33
0.27	0.34	0.36	32	0.26	0.32	0.40	32
0.11	0.08	0.13	31	0.09	0.08	0.13	33
0.04	0.04	0.05	31	0.09	0.14	0.17	31
0.55	0.77	0.92	34.3	0.45	0.65	0.61	
0.04	0.04	0.05	27	0.02	0.05	0.11	
0.18	0.21	0.31	.....	0.15	0.20	0.27	

One outstanding result of the work done this year is the information disclosed as to the great number of methods that passed for the official method. Another interesting point is the fact that some analysts obtained remarkably close check results by either method, while others of equal repute and experience in the analysis of dairy products obtained variations up to nearly 1 per cent. It is surprising, also, to learn that among some chemists check results within 0.05 per cent or less are considered customary and are expected, whereas certain others engaged in similar work and occupying analogous positions consider that a variation of 0.20–0.50 per cent in fat (by difference) is to be expected and in fact is encountered constantly by the analyst.

RECOMMENDATIONS[1].

It is recommended—

(1) That the work on the preparation of butter samples for analysis be continued.

(2) That the official methods of analysis be subjected to collaborative study in comparison with newly proposed methods.

(3) That it be ascertained whether or not tin containers are suitable for butter samples, and can be used in place of glass containers (Mason jars).

No report on cheese was given by the associate referee. A short report of progress is included in the report of the Referee on Dairy Products, and the following paper was submitted.

THE DETERMINATION OF ADDED DISODIUM PHOSPHATE IN PROCESS CHEESE.

By I. M. WILLIAMS (Dairy and Food Commission, Madison, Wisc.).

INTRODUCTION.

Since the issue of the first United States patents in 1916 protecting the manufacture of process cheese the industry has developed to such an extent that the estimated output for 1925 was over 100,000,000 pounds. Process cheese has been defined by legislative action in Wisconsin and Illinois. The Illinois law defines the product as pasteurized or blended cheese rather than process cheese, but in other respects the two laws are practically identical, with the exception of the moisture and fat standards. The amount of emulsifier permissible is not specified in the Illinois law.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1926, **10**: 71.

According to the Wisconsin law process cheese is "the food product produced by mixing, blending and uniting with the aid of heat, cheese of one or more lots of different quality, make, flavor, age, size, weight, shape, of like or different milk fat or moisture content, so as to produce a uniform mass readily makable into desired forms, shapes, sizes, and weights; and may contain added seasoning, added harmless coloring matter, harmless emulsifying agents as disodium phosphate, sodium citrate, sodium and potassium tartrate or mixtures of the same or other harmless emulsifying chemicals in quantities not exceeding three per cent;".

Process cheese can and is being made without the use of emulsifiers, but at the present time most of the manufacturers use them. Of the emulsifiers used, disodium phosphate is the cheapest and most common.

On the basis of some preliminary work done by I. R. Howlett (see Tables 1 and 2) investigating the ratio between the phosphoric acid (P_2O_5) and the calcium oxide of process American cheese containing disodium phosphate as compared with the same ratio of normal American cheese, it was thought advisable to collect sufficient data to determine the value of this ratio in the quantitative examination of process cheese for added phosphate. In an attempt to find a representative value for each kind of cheese, attention was centered on the determination of the P_2O_5/CaO ratio of cheese as found on the market.

Owing to the variation in moisture, fat, and salt content of the individual cheeses, the percentage of phosphoric acid (P_2O_5), total solids, or ash is of little value in the quantitative determination of added disodium phosphate. This point is illustrated by comparing the average phosphoric acid (P_2O_5) content of the sample, total solids, or ash of American cheese, according to Table 1, with the similar values of Sample PA-12, Table 2.

EXPERIMENTAL.

Source of Samples.

The samples, which were representative of the products of a large number of cheese makers, were secured from sources available to the buyer of raw materials for process cheese. They were purchased in the open market, from cheese warehouses, from cheese factories, and from the dairy at the University of Wisconsin. The samples were of different quality, flavor, and fat and moisture content as are the cheeses used in blending to produce a process cheese.

Preparation of Samples.

The cheese samples were thoroughly mixed in a mortar, and samples were taken for the determination of moisture and ash.

Moisture.

A sample of about 3–4 grams of cheese was weighed into a small glass dish containing dried asbestos and a glass stirring rod. The cheese and asbestos were mixed and dried at 110°C. for 5 hours.

Ash.

Twenty-five to thirty grams of cheese was weighed into a platinum dish, and the moisture was driven off by drying overnight at 110°C. The sample was ignited and finally ashed in a muffle at low red heat.

Calcium Oxide.

The ash was treated with nitric acid; the mixture was boiled and filtered; and the filtrate and washings were diluted to 200 cc. Aliquots of 25 cc. were diluted to 40 cc., and the nitric acid was neutralized with ammonium hydroxide. The precipitate that formed was dissolved with citric acid, after which 15 cc. of citric acid solution (70 grams per liter) was added. The calcium was precipitated from the boiling solution with ammonium oxalate, cooled, filtered, ignited, and weighed as calcium oxide[1].

Phosphorus Pentoxide.

Twenty-five cubic centimeters of the nitric acid solution of the ash was diluted to 40 cc., and the phosphorus was determined gravimetrically as magnesium pyrophosphate. To determine whether or not all the phosphorus was retained in direct ashing of the cheese, potassium nitrate and sodium carbonate were incorporated into a sample of each of two cheeses before ashing. A third sample of Cheese 2 was digested with sulfuric acid and potassium nitrate. The results for phosphorus, practically the same by all methods, were as follows:

Percentage of phosphoric acid (P_2O_5) found by different methods.

METHOD OF TREATMENT OF SAMPLE	CHEESE 1	CHEESE 2
Direct ashing	1.49	1.33
Ashing with KNO_3, Na_2CO_3	1.48	1.34
H_2SO_4, KNO_3 digestion		1.36

DISCUSSION OF RESULTS.

The determinations show a range of values for the P_2O_5/CaO ratio for each kind of cheese. The average of this ratio for 28 samples of American cheese is 1.094; for 12 samples of brick cheese, 1.096; and for

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[1] *Z. angew. Chem.*, 1898, **34**: 776.

10 samples of Swiss cheese and one of block Swiss cheese, 1.043. The sample of block Swiss cheese seems to have an unusually high P_2O_5/CaO ratio.

From a quantitative standpoint, the relation that exists between the phosphoric acid (P_2O_5) and the calcium oxide can be used to advantage in determining the amount of added disodium phosphate. The added phosphoric acid is equal to the $P_2O_5(X)$ in the sample minus the product of the $CaO(Y)$ in the sample and the average value for the P_2O_5/CaO ratio (Z) for the kind of cheese under examination.

$$X - YZ = \text{added phosphoric acid } (P_2O_5).$$

Since process American cheese seems to be the most common process cheese on the market, samples of it were secured, and data concerning those that do and those that do not contain added disodium phosphate are given in Table 2, together with the calculated added emulsifier.

The average P_2O_5/CaO ratio of those process cheeses to which no disodium phosphate has been added is 1.076. The difference between this and the average ratio for American cheese according to Table 1 would amount to less than one-tenth of 1 per cent in the calculation of added disodium phosphate. This difference between the average P_2O_5/CaO ratios for American cheese and process American cheese, to which no phosphate has been added, may be due to the use of varying quantities of a cheese that has a low P_2O_5/CaO ratio, such as Swiss cheese, in compounding the product that is sold under the label of pasteurized, blended, or process American cheese.

SUMMARY AND CONCLUSIONS.

1. Values for the ash of the sample and for the phosphoric acid (P_2O_5) and calcium oxide content of the sample, total solids, and ash, and the P_2O_5/CaO ratio were determined on 28 samples of American cheese, 11 of Swiss cheese, 12 of brick cheese, and 13 of process American cheese, some of which contained added disodium phosphate.

2. The average P_2O_5/CaO ratios for each kind of cheese are slightly different.

3. The ratio P_2O_5/CaO is sufficiently uniform in each kind of cheese to be of value in detecting added disodium phosphate quantitatively.

4. By the use of the following equation the added disodium phosphate can be detected quantitatively.

$X - YZ$ = Added phosphoric acid (P_2O_5), in which X is the phosphoric acid present, Y is the calcium oxide present, and Z is the average value of the P_2O_5/CaO ratio for the kind of cheese under examination.

5. The average of the P_2O_5/CaO ratios of the process American cheese analyzed that contained no added disodium phosphate is slightly lower than that obtained as an average for American cheese.

TABLE 1.

Results of analyses of American, Swiss, and brick cheese.

SAMPLE NO.	P_2O_5/-CaO	SAMPLE		TOTAL SOLIDS		ASH		ASH	Na_2HPO_4.-$12H_2O$*
		P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO		
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
AMERICAN CHEESE.									
A-1†	1.023	1.07	1.04	1.78	1.74	32.38	31.64	3.31	−0.3
A-2	1.048	0.99	0.94	1.60	1.53	34.78	33.17	2.85	−0.1
A-3	1.087	1.07	0.99	1.74	1.60	34.79	32.00	3.10	−0.0
A-4	1.090	1.13	1.03			29.51	27.06	3.83	+0.0
A-5‡	1.114	1.06	0.95	1.76	1.58	34.31	30.78	3.11	+0.1
A-6‡	1.101	1.12	1.02	1.89	1.71	38.36	34.83	2.92	+0.0
A-7‡	1.076	1.17	1.08	1.91	1.78	33.26	30.91	3.51	−0.0
A-8	1.083	1.18	1.09	1.95	1.80	36.73	33.88	3.23	−0.0
A-9	1.118	1.11	0.99	1.82	1.63	37.19	33.24	2.99	+0.1
A-10	1.108	1.18	1.06	1.91	1.73	42.10	37.97	2.81	+0.1
A-11	1.036	1.26	1.22	2.00	1.93	34.25	33.05	3.70	−0.3
A-12	1.111	1.10	1.03	1.75	1.57	36.17	32.55	3.17	−0.1
A-13	1.122	1.10	0.98	1.83	1.63	41.28	36.78	2.67	+0.1
A-14	1.210	1.05	0.86	1.78	1.47	45.43	37.53	2.31	+0.5
A-15¶	1.080	1.23	1.14	1.85	1.71				−0.0
A-16¶	1.080	1.09	1.01	1.69	1.58				−0.5
A-17¶	1.180	0.99	0.83	1.61	1.36				+0.4
Maximum	1.210	1.26	1.22	2.00	1.93	45.43	37.97	3.83	
Minimum	1.023	0.99	0.83	1.60	1.36	29.51	27.06	2.31	
Average	1.094	1.11	1.01	1.81	1.66	35.72	32.72	3.15	
SWISS CHEESE.									
S-1**	0.999	1.29	1.29	2.32	2.32	46.72	46.76	2.77	−0.2
S-2**	1.004	1.30	1.29	2.25	2.24	46.33	46.13	2.81	−0.2
S-3**	1.009	1.28	1.27	2.19	2.17	48.02	47.55	2.67	−0.2
S-4**	1.024	1.33	1.30	2.36	2.31	47.65	46.51	2.79	−0.1
S-5**	1.027	1.28	1.24	2.39	2.33	47.68	46.42	2.68	−0.0
S-6**	1.018	1.27	1.25	2.28	2.25	47.27	46.57	2.69	−0.1
S-7**	1.028	1.30	1.27	2.42	2.36	46.53	45.24	2.81	−0.1
S-8††	1.063	1.46	1.37	2.12	2.00				+0.1
S-9	1.036	1.40	1.35	2.28	2.20	42.80	41.28	3.28	−0.0
S-10‡‡	1.060	1.53	1.45	2.32	2.18				+0.1
S-11	1.214	1.46	1.20	2.18	1.79	32.21	26.52	4.54	+1.0
Maximum	1.214	1.53	1.45	2.42	2.36	48.02	47.55	4.54	
Minimum	0.999	1.27	1.20	2.12	1.79	32.21	26.52	2.67	
Average	1.043	1.35	1.29	2.28	2.19	45.02	43.66	3.00	
BRICK CHEESE.									
B-1	1.030	1.16	1.12	1.66	1.61	46.04	44.69	2.52	−0.3
B-2	1.045	1.18	1.13	1.94	1.86	30.04	28.74	3.93	−0.2
B-3	1.032	1.23	1.19	2.09	2.03	42.34	41.03	2.92	−0.3
B-4	1.060	1.26	1.19	1.97	1.86	25.22	23.78	5.01	−0.2
B-5	1.092	1.19	1.09	1.96	1.79	21.07	19.29	5.68	−0.0
B-6	1.131	1.24	1.10	2.00	1.77	35.93	31.74	3.47	+0.2
B-7	1.161	1.20	1.03	1.87	1.61	33.22	28.61	3.62	+0.4
B-8	1.114	1.23	1.10	2.02	1.81	41.45	37.19	2.98	+0.1
B-9	1.106	1.18	1.06	1.92	1.73	35.38	31.97	3.34	+0.1
B-10	1.135	1.12	0.98			28.12	24.76	3.99	+0.2
B-11	1.148	1.21	1.05	1.88	1.64	27.71	24.12	4.37	+0.3
B-12	1.101	1.22	1.11	1.88	1.71	28.22	25.64	4.34	+0.0

TABLE 1.—Continued.

Results of analyses of American, Swiss, and brick cheese.

SAMPLE NO.	P_2O_5/-CaO	SAMPLE		TOTAL SOLIDS		ASH		ASH	Na_2HPO_4.-$12H_2O$*
		P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO		
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
BRICK CHEESE.									
Maximum	1.161	1.26	1.19	2.09	2.03	46.04	44.69	5.68	
Minimum	1.030	1.12	0.98	1.66	1.61	21.07	19.29	2.52	
Average	1.096	1.20	1.09	1.92	1.76	32.89	30.00	3.84	

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* Percentage error calculated by using the average P_2O_5/CaO ratio of the American, Swiss, and brick cheeses, respectively, rather than the actual P_2O_5/CaO ratio of the individual cheese.
† Composite of three samples.
‡ Composite of four samples.
¶ I. R. Howlett.
** Unsalted.
†† Imported Swiss cheese.
‡‡ Block Swiss cheese.

TABLE 2.

Results of analysis of process American cheese with and without disodium phosphate.

(Containing no added disodium phosphate.)

SAMPLE NO.	P_2O_5/-CaO	SAMPLE		TOTAL SOLIDS		ASH		ASH	Na_2HPO_4-$12H_2O$*
		P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO		
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
PA-1	1.066	1.04	0.97	1.59	1.49	19.72	18.49	5.29	−0.1
PA-2	1.061	1.17	1.10	1.73	1.63	24.55	23.12	4.76	−0.1
PA-3	1.114	1.05	0.94	1.70	1.52				+0.1
PA-4	1.073	1.02	0.95	1.64	1.53	25.54	23.79	4.02	−0.1
PA-5	1.058	0.99	0.93	1.62	1.53	19.73	18.63	5.04	−0.1
PA-6	1.087	1.03	0.95	1.72	1.58	25.98	23.88	3.99	−0.0
PA-7	1.073	1.07	1.00	1.78	1.66	24.95	23.24	4.32	−0.1
Maximum	1.114	1.17	1.10	1.78	1.66	25.98	23.88	5.29	
Minimum	1.058	0.99	0.93	1.59	1.49	19.72	18.49	3.99	
Average	1.076	1.05	0.97	1.68	1.56	23.41	21.85	4.57	
(Containing added disodium phosphate.)									
PA-8	1.721	1.53	0.88	2.55	1.48	33.06	19.19	4.63	+2.9
PA-9	1.599	1.72	1.07	2.55	1.59	30.49	19.04	5.64	+2.8
PA-10	1.964	1.68	0.85	2.78	1.41	31.39	15.98	5.36	+3.8
PA-11	1.741	1.63	0.94	3.19	1.83	33.15	19.03	4.94	+3.1
PA-12	1.651	1.94	1.17	3.27	1.98	33.88	20.52	5.73	+3.3
PA-13	1.400	0.82	0.58	1.60	1.14	21.12	15.04	3.87	+0.9
PA-14†	1.620	1.57	0.96	2.65	1.67				+2.6
PA-15†	1.600	1.70	1.06	3.14	1.96				+2.7
Maximum	1.964	1.94	1.17	3.27	1.98	33.88	20.52	5.73	
Minimum	1.400	0.82	0.58	1.60	1.14	21.12	15.04	3.87	
Average	1.662	1.57	0.94	2.71	1.63	30.51	18.13	5.03	

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* Percentage error calculated by using the average P_2O_5/CaO ratio of all process American cheese rather than the actual P_2O_5/CaO ratio of the individual cheese
† I. R. Howlett.

REPORT ON DRIED MILK.

By J. T. KEISTER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The time of the associate referee was devoted to: (1) The further study of the determination of moisture by the vacuum-oven method, upon which report was made last year[1]; and (2) the study of the present tentative method for the determination of fat[2].

Three samples, representing (1) a "spray-dried" whole milk powder, (2) a "drum-dried" partially skimmed powder, and (3) a skimmed powder (method of drying not known), were submitted to four collaborators. The methods used in the work follow:

MOISTURE IN MILK POWDER.

APPARATUS.

(a) *Aluminum dish.*—Diameter 55 to 60 mm. and about 15 mm. deep, provided with a slip-in inverted cover fitting tightly on the inside.

(b) *Vacuum oven.*—Should have a vacuum of at least 25 inches or not exceeding 5 inches' pressure.

TABLE 1.

Moisture and fat in milk powders—results by collaborators.

COLLABORATOR	WHOLE MILK			PARTIALLY SKIMMED			SKIMMED		
	Moisture		Fat	Moisture		Fat	Moisture		Fat
	Dried 3 hours	Dried 5 hours		Dried 3 hours	Dried 5 hours		Dried 3 hours	Dried 5 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Fred Hillig	2.55 2.50	2.57 2.49	26.69 26.77 26.81 26.82	4.64 4.63	4.78 4.75	11.47 11.48 11.59	8.15 8.12	8.23 8.23	0.99 0.93 0.95
L. H. Bailey	2.38	2.44 2.48	25.70 25.71 25.76	4.59 4.61	4.72 4.58	11.31 11.42	8.12 8.14	8.27 8.31	0.83 0.83
C. E. Goodrich	2.66 2.69	2.77 2.69	26.65 26.79	4.84 4.76	4.81 4.80	11.49 11.36	8.27 8.29	8.56 8.56	0.90 1.02
C. A. Greenleaf	2.52 2.52	2.64 2.64	27.20 27.18	4.75 4.63	4.77 4.72	11.32 11.27	8.38 8.17	8.49 8.38	0.95 0.94
J. T. Keister	2.54 2.47	2.63 2.67	27.05 27.12	4.87 4.92	4.73 4.75	11.95 11.81	8.17 8.07	8.31 8.37	0.89 0.85
Maximum Minimum Average	25.70 27.20 } 1.50			11.27 11.95 } 0.68			0.83 1.02 } 0.19		

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[1] *This Journal*, 1926, 9: 243.

[2] *Ibid.*, 1925, 8: 482.

DETERMINATION.

Weigh about 1 gram of the well mixed sample free from lumps into a previously weighed dish, leaving cover on during weighing. Place the dish in direct contact with the metal shelf of the vacuum oven. Remove the cover and dry for 5 hours under a pressure not exceeding 5 inches (25 inches' vacuum) at the temperature of boiling water. During the drying, admit into the oven a slow current of air (about 2 bubbles per second), drying by passing through concentrated sulfuric acid. Turn off vacuum pump and slowly admit air into the oven. Press the cover tightly into the dish while still in the oven, place in a desiccator, cool, and weigh. Express loss in weight as moisture.

FAT IN MILK POWDER—TENTATIVE METHOD[1].

Weigh out about 1 gram of well mixed sample into a small lipped beaker. Add about 1 cc. of water. Mix well with a glass rod to form a thick liquid free from lumps. Add

TABLE 2.

Moisture in milk powders—results by associate referee.

TYPE OF POWDER	DRYING FOR 3 HOURS IN VACUUM AT 97°-100°C	DRYING FOR 5 HOURS IN VACUUM AT 97°-100°C.	DRYING IN WATER OVEN AT 100°C.
	per cent	*per cent*	*per cent*
Skim milk	3.83 3.82	3.87 3.82	
Skim milk	4.60 4.50	4.83 4.81	
Skim milk	6.18 6.05	6.29 6.26	
Skim milk	5.90 5.90 (6.08)	5.86 5.86 (6.02)	
Partly skimmed "drum process"	2.69 2.68	2.74 2.79	1.96 1.98
Skim milk	2.77 2.69 2.71	2.79 2.81	2.04 1.94
Skim milk "Just" process (drum process)	3.46 3.44	3.41 3.46	3.24 2.92
Skim milk "Just" process	3.84 3.79	3.82 3.84	
Skim milk	4.82 4.81	4.87 4.85	
Whole milk "spray" process	1.64 1.74 1.75	1.67 1.73 1.69	
Skim milk	4.11 4.12	4.16 4.20	
Whole milk "spray" process	2.49 2.58	2.67 2.64	

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[1] *Ibid.*, 1925, 8: 482.

9 cc. more of water and 1 cc. of ammonia. Warm on the steam bath to 50°–60°C. Transfer to a Röhrig tube or similar apparatus. Cool. Add 10 cc. of 95 per cent alcohol. Mix. Add 25 cc. of ethyl ether and proceed with the extraction as in the official Roese-Gottlieb method for milk. Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present. In case of whole milk and cream powders, make a third extraction, using a mixture of 15 cc. of each ether.

The results reported by the collaborators are presented in Table 1. In Table 2 the results obtained independently by the associate referee are given.

The following comments were made by collaborators on the moisture work:

COMMENTS OF COLLABORATORS.

L. H. Bailey: The three hours' drying under the conditions outlined in the method is insufficient to secure complete dryness.

C. E. Goodrich: In case of the "part skim" product, the three hours' drying period seems comparable with the five hour period. The other samples were not so completely dried after three hours, the greatest variation being with the skimmed powder.

The following comments on the determination of fat were made by the collaborators:

F. Hillig: It would seem that specific instructions as to temperature and length of time for warming on the steam bath should be inserted in the method.

L. H. Bailey: The fats were dissolved in chloroform instead of petrolic ether as provided for in the method.

C. E. Goodrich: Same comments as given by Bailey.

DISCUSSION.

The results obtained on moisture, Table 2, confirm the conclusions of the associate referee given last year. They show that at least three hours' drying in vacuo at a temperature of nearly 100°C. is required for the complete removal of the moisture from "milk powders" in any case and that in some instances an additional two hours is indicated. This conclusion is in accord with the findings of the collaborators as shown in Table 1. The results obtained by the collaborators are very satisfactory from the standpoint of checks. A slight darkening of the desiccated material was noted, which would indicate decomposition. It would seem desirable to make a comparative study of this method and the Bidwell-Sterling procedure. The latter method, it is understood, removes the moisture without decomposing the material.

As regards the data on the determination of fat, it may be said that the check determinations of the individual analysts are acceptable in all instances, and for the most part they are very close. This would indicate that the procedure is satisfactory, and that the discrepancies are due either to a difference in the sample or to a lack of specific details in the directions of the method. That the difficulty is not due to a difference in the sample is indicated by the fact that the low results by Bailey and the results of Goodrich (which agree well with the other collaborators) were obtained on the same subdivision of the sample. It is believed that the directions for the method which relate to the extraction of fat are clearly stated and it only remains to consider the preparation of the sample for extraction. It was found that 1 cc. of water is not always sufficient to permit of the preparation of a smooth paste; about 2 cc. is necessary for some samples. Also the directions for warming the sample on the steam bath are too indefinite. It is believed that the changes in the directions dealing with the latter point are desirable and should be written into the method. The time and temperature to be used should be made the subject of study.

RECOMMENDATIONS[1].

It is recommended—

(1) That the method here described for determining moisture in milk powders be compared with the Bidwell-Sterling method before finally adopting it as a tentative method.

(2) That the tentative method for the determination of fat in milk powders be further studied with a view to establishing the best conditions of preparing the sample for extraction.

(3) That the method for preparing sample and the determination for protein and ash as given under "Malted Milk", page 275, **61**, **63**, and **64**, be adopted tentatively for "dried milk". These methods are generally recognized and are applicable to such products; however, it is suggested that "as prepared for sale" be deleted from **61**.

REPORT ON MALTED MILK.

By B. G. Hartmann[2] (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

No work was undertaken on the studies of methods for the analysis of malted milk recommended at the last meeting of the association[3].

In view of the fact that there is some question regarding the adequacy of the definition contained in Circular 136[4], particularly that part of it which pertains to "the full enzymic action of the malt extract", it was believed advisable to identify the product before considering methods of analysis. Furthermore, the Food Standards Committee directed that a study of the enzymic activities of malt on milk be made so that the necessary information might be available if it was found desirable to revise the definition for malted milk. Accordingly the entire time of the associate referee was devoted to this question.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 72

[2] Presented by F. Hillig.

[3] *This Journal*, 1926, **9**: 80.

[4] U. S. Dept. Agr. Circ. 136.

The following is a brief statement of the results obtained on the enzymic activities of malt on milk.

Malted milk is essentially the product resulting from evaporation in vacuo of an especially prepared malt-flour infusion and milk. Although the methods used in different factories vary somewhat with respect to the quantities of malt and flour used, and also in the manner of preparing the mash, the final products are very much the same from the standpoint of chemical composition. Biological differences may exist, since in the process of manufacture the temperatures and the manner of adding the alkali for neutralization have a marked effect on enzymic activities. There is no doubt that in the absence of careful control the action of enzymes will be decreased or even inhibited.

The first step in the investigation was directed to determining the best conditions for obtaining maximum enzymic activities. Since the malt infusion obtained from the malt-flour mash is the enzyme carrier it is obvious that means for preserving its enzymic content during manufacture are of first importance. The mashing process was studied particularly with regard to conditions of temperature and time required for obtaining a suitable extraction of the malt-flour mixture. A high diastatic malt and a good grade of wheat flour were used. The mash was prepared by gelatinizing the flour with the aid of heat and adding an equal quantity of freshly ground malt and sufficient water to yield an infusion of a solid content of approximately 18.5 per cent when mashed at 65°C.

The following points relating to the effect of temperature and hydrogen-ion concentration during mashing were established:

1. A malt-flour infusion prepared at 65°C. (the temperature generally used in practice) has a pH of about 5.7 and an acidity in terms of lactic acid of approximately 0.14 per cent.

2. The mashing temperature does not materially affect the hydrogen-ion concentration; however an increase in acidity is obtained at lower mashing temperatures.

3. The highest sugar degree (percentage of sugar in solids) is obtained at a temperature of 55°C. However, for complete conversion of starch a temperature of 65°C. is necessary.

4. The temperature of 55°C. does not permit of complete starch conversion in 4 hours, but if the temperature is increased to 65°C. for 1 hour a maximum sugar degree is obtained.

Having established the characteristics of the malt infusion, the next step was to determine its biological effect upon milk. This phase of the work was restricted to the study of the effect of the more important enzymes, lipase and proteases.

For following the action of lipase digestion, measurements of acidity increases were almost wholly relied upon. For recognizing proteolytic activities determinations of amino nitrogen and soluble protein were made. Measurements of viscosity, hydrogen-ion concentration, and titratable acidity were also included. It is not necessary to go into details regarding the data obtained. It seems sufficient at this time to give a brief summary of the main features of the work.

LYPOLYSIS.

For determining lypolysis, malt infusions prepared at temperatures ranging from 35° to 65°C. were permitted to act upon neutral butter fat and dried whole milk at pH ranges of 5.0, 5.8 and 6.5. Broadly speaking, no increase in acidity was observed after 4 hours of incubation in any of these experiments. There were indications in some instances of a slight increase or decrease in acidity, but these were not pronounced enough to justify the conclusion that these changes were caused by lipolytic activity. After 20 hours of incubation a decided increase in acidity was observed in some cases, particularly at low temperatures, that is between 35° and 45°C. This increase was due to bacterial action.

From these results it was concluded that no lipolytic action occurs when malt infusions prepared at temperatures of 35° to 65°C. and at pH values of 5.0, 5.8, and 6.5 are permitted to act on neutral butter fat and dried whole milk. Since these temperatures and pH ranges cover the conditions generally obtaining in good factory practice it is very doubtful that lipolytic digestion of fats occurs during drying in the manufacturing process.

STUDIES OF PROTEOLYTIC DIGESTION.

Studies of proteolytic digestion were conducted in very much the same manner as the experiments just described. However, in this instance the digestion was measured by the increase of the amino nitrogen and of the soluble protein that occurred during the incubation of the various mixtures. It was found impracticable to use fresh whole milk. Difficulty was also experienced in using dried whole milk, because it was found impossible to obtain the necessary clear filtrates for the various determinations. After unsuccessful attempts to use dried whole milk, milk proteins precipitated from dried whole milk with 70 per cent alcohol were employed. Additional experiments were also made with pure casein. The digestions were conducted at carefully controlled temperatures ranging from 35° to 65°C. and pH ranges of 5.0 to 6.5. The mixtures were incubated for 4 hours and 20 hours. For determining the amino nitrogen content the Van Slyke method was followed. The soluble proteins were determined by digesting the material according to the modified Kjeldahl-Gunning-Arnold method. Determinations of viscosity, acidity, and hydrogen-ion concentration were also made.

The results of determinations of the amino nitrogen and soluble protein content showed an increase after 4 hours and 20 hours of incubation at all the temperatures and for all the hydrogen-ion concentrations used. These results showed also that the most active digestion usually occurred at a pH of 5.0 and a temperature between 45° and 55°C. The changes in viscosity were marked, decreasing as the length of the incubation period increased. These changes are due to an increase in the sugar content of the mixtures rather than to digested protein. The acidities showed a slight increase in all cases.

It was also deemed advisable during these experiments to ascertain just what changes take place in the malt infusions themselves during incubation. It was found that at low temperatures there is a considerable change in the amino nitrogen content during incubation at a pH of 5.0. The largest increase took place at 35°C. and gradually decreased until for 55° and 65°C. no changes were detected. Using the determinations on the malt infusions themselves as blanks and deducting the results from the totals obtained in the digestion experiments on milk protein and casein, it was found that a positive increase in amino nitrogen and soluble protein occurred due entirely to the digestion of the milk protein and casein. One of the striking developments in the investigation was the uniform relation existing between the increase in amino nitrogen and the acidity.

During the evaporation process in factory practice a vacuum of 27 inches is maintained, which corresponds to a temperature of approximately 45°C. This is the temperature found in this investigation for optimum proteolytic activity.

CONCLUSIONS.

From the results obtained in this investigation it is concluded that with proper control of conditions with respect to temperature and hydrogen-ion concentration, a malt infusion will cause digestion of milk proteins. Furthermore, it is concluded that there is no digestion of fats through lipolytic activities during the process of manufacture.

RECOMMENDATION[1].

It is recommended that the study of methods proposed last year be continued during the coming year.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 72.

REPORT ON ICE CREAM[1].

By L. H. McRoberts[2] and R. E. Remington[3].

The present associate referee was appointed in June of this year. Although some work has been done on two different methods (the gelatin and ash content) the investigation has thus far been confined to his laboratory.

The instructions to the former associate referee, A. C. Dahlberg, to "give special attention to methods for the determination of sugars, milk solids, and gelatin" were noted, with the result that particular attention was given to a method for gelatin. The present official methods for the determination of ash in milk, cream, condensed milk, and sweetened milk were also investigated with reference to formulating a method for ash in ice cream.

R. E. Remington was employed by this department during the summer months and is responsible for a large part of the work in investigation of methods of analysis for ice cream.

Although no collaborative work has been done on the two methods here considered, it is believed that a trial of the method for ash will meet with favor. As noted in the description of the proposed method for gelatin, certain points required further investigation before it could be submitted with the recommendation of the associate referee as a satisfactory method for food inspection analysis.

THE DETERMINATION OF GELATIN IN ICE CREAM.

It is believed that the importance of the determination of gelatin in ice cream lies not so much in finding the actual gelatin content as in making possible a means of calculating the milk solids not fat. Dahlberg mentions the possibility of determining milk solids as based on their nitrogen content and states in his recent correspondence: "Many states have laws governing the total solids not fat that can be put into ice cream. Most of these laws require not less than 10 per cent of milk solids not fat, although there is no official method of making this determination. I recognize that such a determination may be an absolute impossibility, yet if this is true and there is some definite relationship between some of the constituents, such as the proteins and the milk solids not fat, it may be possible to bring out this fact and have a method for making a protein determination. It may even be desirable to bring this inconsistency to the attention of interested people with the idea of having an enforceable standard used in the future".

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[1] Presented by J. Hortvet.

[2] Associate Referee (Food and Drug Laboratory, Bismarck, N. D)

[3] North Dakota Agricultural College, Fargo, N. D

A determination of the total nitrogen content of ice cream will, of course, include gelatin; therefore, if the nitrogen figure is to be of value a correction must be made for the gelatin content.

Since the ash content of normal milk averages very close to 0.7 per cent it is thought that a nitrogen ash ratio correction for gelatin will be of value in calculating milk solids and in the detection of added neutralizers.

Ferris[1] has reported determinations of gelatin on a number of ice cream mixes and with very good results, so that it was thought advisable to make a thorough trial of the suggested method before attempting to formulate a new method. In earlier trials by this method the associate referee always obtained low results, usually recovering about 90 per cent of the gelatin added. By certain modifications it was possible to obtain a more complete recovery, but still not all the gelatin added.

In order that the discussion and suggested changes may be followed to best advantage the Ferris method will be given in detail and then the method modified as believed necessary for food inspection analysis.

GELATIN.

Ferris Method.

Weigh 450 grams of ice cream into a 500 cc. flask, warm to 35°C., and dilute to the mark. Transfer to a larger flask and add 10 per cent acetic acid until the casein is coagulated, observing the volume of acetic acid added. Calculate the volume of the fat and casein (volume of fat = weight of fat in sample × 1.075; volume of casein = weight of casein in sample × 0.73), and determine the volume of serum by subtracting this from 500 plus the number of cc. of acetic acid added. If the curd does not settle readily, shake with a small quantity of carbon tetrachloride, centrifuge, and decant the supernatant serum. Measure 100 cc. of the serum into a 400 cc. lipped beaker, and while still warm add slowly, with stirring, 200 cc. of 95 per cent alcohol. Cool in ice water and let stand until the precipitate settles, leaving the supernatant liquid clear. (At this point the precipitate may be allowed to settle overnight in an ice box at as near zero Centigrade as possible.) Filter on a large hardened paper in a Büchner funnel and wash with cold alcohol, two volumes of 95 per cent alcohol, and one of water. Drain the precipitate, remove to a small beaker, and rub up with 50 cc. of water. Allow to soak at room temperature until the gelatin swells, then heat to about 90°C. in a hot water bath, filter, and wash with hot water. Make up to 100 cc. at 35°C. and polarize. Cool a portion of the solution rapidly to 15°C., pour into a cold polariscope tube, let stand at 15°C. for 24 hours, and polarize again. The reading at 15°C., divided by the reading at 35°C., will give an indication of the jelly strength of the gelatin[1]. Calculate the percentage of gelatin in the sample from the reading at 35°C. from the following formula:

$$\text{Percentage of gelatin} \quad \frac{R \times 0.346 \times V \times 100}{W \times 2 \times \text{specific rotation}}.$$

When W = weight of sample; V = volume of acetic acid serum; and
R = polariscope reading in Ventzke degrees in a 2 dm. tube.

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[1] *J. Dairy Sci.*, 1922, **5**: 555.

The specific rotation of high-grade gelatin is given by Smith[1] as 141, when calculated to a moisture and ash-free basis. Determine nitrogen in an aliquot of the gelatin solution and calculate the percentage of gelatin in the ice cream as follows:

$$\text{Percentage of gelatin} = \frac{N \times V \times 5.55 \times 100}{a \times W},$$

When W = weight of sample; V = volume of acetic acid serum; a = volume of aliquot of gelatin solution; and N = weight of nitrogen in aliquot of gelatin solution.

Modified Method.

Into a tared 400 cc. beaker weigh 200 grams of the melted and thoroughly mixed sample. Add 25 cc. of water and enough hematoxylin indicator to color the sample distinctly pink. Heat to 40°C. and titrate with 10 per cent acetic acid until the pink color has completely disappeared. Return the beaker to the balance and add water until the weight of the contents reaches 250 grams. Accuracy of 0.5 gram is sufficient. Mix thoroughly and return to the 40°C. water bath until the curd has fully separated. Filter through a small bag of cotton or linen.

Weigh out 100 grams of the filtrate, add 3 cc. of a saturated solution of potassium alum, then 200 cc. of 95 per cent alcohol, with stirring. (Cooling hastens the separation of the precipitate.) Filter by suction on a 9 cm. filter, using a Büchner funnel. Tear up the paper and place in water in a small beaker. Soak until the gelatin has an opportunity to swell, then place the beaker in warm water for half an hour, first adding water to approximately 50 cc. Bring rapidly to incipient boiling and filter at once into a 100 cc. volumetric flask, washing with hot water until the flask is nearly full. Allow the flask to cool to near room temperature, make to the mark, and mix.

Make the polarization at once, using a jacketed tube. Circulate water, that has been heated, through the jacket until a thermometer placed in the tubulature reads 40°C. Then shut off the water, allow the tube to cool to 35°C., and read. If the flasks are not read at once, heat them in a water bath at 35–40°C. for half an hour or more before placing in the polariscope tube.

The weight of serum to be used as a basis in calculating is obtained by subtracting the weight of fat and casein in 200 grams of the sample from 250 grams.

By adhering strictly to the quantities given in the modification of the Ferris method the calculation becomes much simplified. The percentage of gelatin is obtained by substituting the weight of the serum for the volume in the original formula. But since the total weight of the sample is 200 grams and the specific rotation taken is 117 for commercial gelatin, the only variables become the polariscope reading (R) and the weight of the serum (W), and hence:

$$\text{Percentage of gelatin} = 0.00074 \times R \times \text{W}.$$

In the estimation of gelatin in the final solution on the basis of the nitrogen content the factor 6.75 is recommended instead of 5.55, as used by Ferris, and the formula therefore reduces to:

$$\text{Percentage of gelatin} = 0.135 \times N \times W,$$

in which N = mg. of nitrogen in 25 cc. of final solution and W = the weight of the serum.

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[1] *J. Am. Chem. Soc.*, 1919, **41**. 135; *J. Ind. Eng. Chem.*, 1920, **12**: 878.

It is always necessary to correct the value (*N*) for nitrogen found in blank determinations. That the filtrate actually does contain proteins other than gelatin is shown by the invariable appearance of a beautiful violet color (tryptophane reaction) as soon as the sulfuric acid is added for digestion. Pure gelatin becomes yellow, but never shows violet.

SUGGESTED CHANGES IN THE FERRIS METHOD FOR THE QUANTITATIVE DETERMINATION OF GELATIN IN ICE CREAM.

(*1*) *Smaller Sample.*

Commercial ice cream weighs from 500 to 600 grams to the frozen quart, with an average weight of possibly 550 grams. Because a quart makes a convenient sample, it appeared desirable to so modify the method as to permit the use of a smaller quantity, that duplicate determinations might be made from one quart and sufficient material remain for other analyses. Therefore a sample of 200 grams instead of 450 grams is suggested.

(*2*) *Basis of weight dilution instead of volume dilution.*

Ice cream contains a large amount of air incorporated throughout the mass in finely divided form; owing to the colloids present this air escapes very slowly, even when the sample is melted. Many samples, particularly those that contain milk powder, in which the casein is but imperfectly brought into solution, will on melting separate a frothy curd from which the air cannot be well removed. A method involving dilution to definite volume is thus open to criticism when applied to commercial ice cream.

(*3*) *Alum solution used with alcohol to give more complete precipitation of gelatin.*

In attempting to account for low results it was noted that when two volumes of alcohol were added to a gelatin solution, no definite precipitation was produced, even at 0°C., if the gelatin solution was of 0.5 per cent concentration, and at 1.0 per cent only a part of the gelatin was precipitated in visible form. The addition of a polyvalent cation should help, and actually, a few cc. of a saturated solution of alum, with the alcohol, precipitated the gelatin promptly and completely at room temperature. Alum alone does not precipitate isoelectric gelatin, nor does alcohol alone, but the two together insure a quantitative precipitation.

(*4*) *Procedure with reference to the final solution of gelatin.*

The precipitate obtained with alum and alcohol is heated to 90°C. after treatment with water—not only to dissolve the gelatin, for which no such high heat is needed or desired, but to coagulate the albumin that was not precipitated with the casein in the first separation. Consequently, bringing the beaker up to this temperature as rapidly as possible and filtering at once is favored and recommended.

For extreme accuracy the final dilution should be made at the temperature at which the specific rotation of gelatin was determined and at the temperature at which the solution is to be polarized. Practically making to volume at 20°C. introduces an error of only 0.4 per cent of the gelatin present.

To insure that the gelatin is all in the sol form, it is held at a temperature slightly above 35°C. for some time before polarizing.

(5) *Specific rotation values of commercial gelatin.*

In arriving at the value which he used for the specific rotation of commercial gelatin (−122), Ferris is evidently guided by the data on four samples, of which he does not give the origin, and by the work of Smith, to which reference has been made, who used only six samples, mostly of imported gelatin. Smith calculated from his observed rotation of −123 that the rotation on a moisture- and ash-free basis should be −141, assuming for this purpose a content of 11.4 per cent moisture and 1.6 per cent ash. Bogue[1], however, reports that the moisture content varies with the humidity and with the grade of the material, higher grade products being more hygroscopic. For medium humidity and best grades he finds 15–16 per cent of moisture. If −141 is the correct rotation for pure dry gelatin, then −123 is too high for gelatin containing over 13 per cent of moisture.

Obviously, if the method is to be applied in inspection analysis and the analyst has no means of knowing the constants on the particular lot of gelatin used in the ice cream, accurate results cannot be assured unless it is possible to deduce an average value for the rotation which can be applied without too great an error to any grade of gelatin likely to be encountered.

In order to obtain such average values of specific rotation of commercial gelatin, samples were obtained from ice cream makers in the State of North Dakota and also from manufacturers of gelatin catering to the ice cream trade. On these samples determinations for moisture, ash, nitrogen, and specific rotation were made. The results are given in Table 1.

The results given in Part 1 of the table are doubtless more reliable than those given in Part 2, because the samples came directly to the associate referee from makers of gelatin and without the possibility of contamination that exists when the packages are opened in ice cream establishments. The average value for the specific rotation of commercial gelatin was, however, the same on both lots (−117°). It is recommended, therefore, that the value of *−117* be used in the calculation when gelatin is determined by polarization.

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[1] Chemistry and Technology of Gelatin and Glue. McGraw-Hill, 1922.

TABLE 1.

Results of analyses.

SAMPLE NUMBER	MOISTURE	ASH	NITROGEN	ROTATION IN 1 PER CENT SOLUTION AT 35°C.	ON DRY, ASH-FREE BASIS	
					NITROGEN	ROTATION
	per cent	*per cent*	*per cent*		*per cent*	
Part 1.—Gelatin used by ice cream makers in North Dakota.						
6054	13.03	1.61	15.26	123	17.88	144
6055	14.48	1.47	14.95	114	17.78	136
6056	14.50	1.58	14.87	114	17.72	136
6057	14.31	0.86	15.28	121	18.02	143
6058	12.76	1.97	15.26	118	17.89	138
6059	14.74	2.51	14.61	116	17.65	140
6062	13.81	1.00	15.23	118	17.88	139
6070	15.25	1.58	14.50	119	17.43	143
6071	13.17	1.53	14.96	122	17.54	143
6072	12.73	0.98	15.30	124	17.73	144
6073	15.53	2.05	14.20	114	17.23	139
6074	15.58	3.12	14.27	108	17.56	133
6075	14.68	2.34	14.67	111	17.67	134
Highest	15.58	3.12	15.30	124	18.02	144
Lowest	12.73	0.86	14.20	108	17.23	133
Average	14.20	1.74	14.81	117	17.69	139
Part 2.—Ice cream gelatins from manufacturers.						
6065	12.45	0.74	15.36	122	17.69	141
6066	11.71	0.75	15.20	119	17.37	136
6067	13.30	0.97	15.20	119	17.73	139
6068	14.96	2.11	14.59	114	17.59	138
6069	14.69	2.24	14.66	114	17.64	138
6076	13.93	2.60	14.64	115	17.54	138
6077	13.23	2.31	14.65	116	17.35	137
6078	13.01	2.02	15.02	119	17.67	140
6079	13.17	2.16	14.74	119	17.40	140
6080	14.64	2.25	14.39	115	17.31	139
6081	15.33	1.12	14.65	117	17.53	140
6082	13.73	1.02	14.93	118	17.63	138
6083	14.08	1.10	14.99	117	17.67	138
6084	13.40	1.35	15.02	118	17.62	138
6085	13.20	2.21	14.60	118	17.26	139
Highest	15.33	2.60	15.36	122	17.73	141
Lowest	11.71	0.74	14.39	114	17.26	136
Average	13.66	1.66	14.84	117	17.53	139

(*6*) *Nitrogen factor to be used when gelatin is estimated on nitrogen basis.* In calculating gelatin from the nitrogen determinations, Ferris made use of the nitrogen values of the individual samples of gelatin used, viz., 15.7 per cent for three samples and 14.8 for the fourth. In his method, however, he directs the use of the factor 5.55, which corresponds to 18.0 per cent of nitrogen. Smith prepared ash-free gelatin and found it to contain 17.53 per cent of nitrogen on the dry basis, which is quite comparable with the results of Halla[1] and Bogue, reference cited, 17.61 and 17.5, respectively. But if 17.5 is the correct nitrogen value, then

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[1] *Z. angew. Chem.*, 1907, **20**: 24.

5.71 is the factor, while if it is desired to calculate gelatin on the basis of the original commercial material a still higher factor should be used. On the basis of the samples analyzed and listed in Table 1 the factor *6.75* is recommended to be used in the calculation of commercial gelatin from the nitrogen determination.

(*7*) *Blank determination necessary.*

Ferris does not specify any correction for nitrogen in the final filtrate that is not due to gelatin. From 2 to 3 mg. of nitrogen has been found in 25 cc. of the final solution in blank determinations containing no gelatin. Although good results may be obtained by the Ferris method as here modified, great care is needed in order to achieve success. No mention was made in the discussion of the use of an indicator as noted in the precipitation of the casein. Best results were obtained with hematoxylin to indicate the complete precipitation of casein and fat, but even with this indicator experience with the method is necessary to obtain comparable results.

Further investigation will be made in an effort to standardize certain points with reference to precipitations, separations, and figures to be used in the calculations before submitting the method to collaborators.

THE DETERMINATION OF ASH IN ICE CREAM.

Ice cream may be said to resemble cream in its high fat content and sweetened condensed milk in that a large part of its dry matter is sugar. The methods of the A. O. A. C. for the ashing of milk, cream, and sweetened condensed milk were therefore investigated as to their applicability to ice cream.

In attempting to evaporate to dryness after the addition of strong nitric acid, as in the method for ash in milk and cream, considerable effervescence resulted, and extreme care was necessary to avoid a large loss. Direct ashing after evaporating the sample to dryness without the use of nitric acid is also open to objection in that charring of the sugars produces some water, which, on account of the fat present, is apt to cause spattering.

These difficulties might be overcome if the ash determination were to be made on the residual solution from the fat determination by the official Roese-Gottlieb method[1] for ice cream. This should give reliable results if (1) no mineral matter is carried away in the ether layer, and (2) no mineral matter is introduced in the ammonia or by the action of ammonia on the tubes used for the extraction. Blank determinations have satisfied the associate referee that these conditions have been fulfilled when Pyrex tubes are used. Instead of the Röhrig tubes usually used for the fat determination, Pyrex tubes of 25 x 250 mm. dimensions, equipped with devices for blowing off the ether similar to those employed in the Werner-Schmidt method[1] are preferred.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 89.

The suggested method for the determination of ash in ice cream is as follows:

ASH IN ICE CREAM.

After the last portion of the ether has been removed in the determination of fat, place the tubes in a water bath and heat slowly until the ether and most of the alcohol have been expelled. (Frothing is kept down by a jet of air directed into the tube.) Pour into a weighed dish, washing the tube twice with small quantities of water, and evaporate to dryness on the water bath. Ignite and weigh as usual.

Care should be taken in igniting the residue, but there should be no difficulty because all the fat has been removed. It is easily possible to ash milk products rapidly at temperatures below 500°C. The usual directions to ash at dull redness, or below redness, are vague, as pointed out by Hopper[2], because this point would depend on the eye of the observer and the illumination of the room. Pyrometer control would insure a definite temperature and was used in the ashing of the milk products listed in Table 2.

TABLE 2.

Results of a comparison of methods for ashing of dairy products.

SAMPLE	WITH NITRIC ACID	WITHOUT NITRIC ACID	WRITER'S METHOD
	per cent	*per cent*	*per cent*
1 Milk	0.71	....	0.71
2 Milk	0.72	0.73	0.73
3 Milk	0.68	0.69	0.69
4 Cream	0.54	.	0.56
5 Cream	0.56	0.52	0.58
6 Ice cream	0.54	..	0.64
7 Ice cream*	0.55	0.55	0.55
8 Ice cream	0.54	0.54	0.57

* This sample was ashed with extreme care.

The suggested method is evidently as accurate as either of the former methods when applied to milk and gives higher results when applied to cream and ice cream, thereby accounting for the loss due to spattering. Sample No. 7 was ashed with extreme care by all the methods, which accounts, no doubt, for the identical results. However, more time was needed in the case of the former methods than can usually be given to routine examination.

SUMMARY OF REPORT.

Determination of Gelatin in Ice Cream.

Changes are suggested in the Ferris method for the estimation of gelatin in ice cream that make the method more accurate and better adapted for use in inspection analysis. These are (1) smaller sample, (2) weight instead of volume dilution, (3) control of the casein precipitation by means of hematoxylin indicator, (4) the use of alum to assure complete precipitation of the gelatin, and (5) the correction of the nitrogen found for that from the milk which comes through into the filtrate.

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[1] Leach. Food Inspection and Analysis, 1920, p. 126.

[2] Agricultural Experiment Station, Fargo, N Dak. Personal communication.

Nitrogen determinations and specific rotation values have been obtained for 28 samples of commercial ice cream gelatin, the average value of the nitrogen being 14.84 and for the specific rotation 117, corresponding to 17.53 and 139 on the dry, ash-free basis. These values are recommended to be used in calculating gelatin determinations in ice cream.

Determination of Ash in Ice Cream.

1. The official method for the determination of ash in milk is criticised, particularly when applied to ice cream, on account of the liability to mechanical loss through the effervescence due to production of carbon dioxide from the action of nitric acid on sugar.
2. The official method for ash in condensed milk is not applicable to ice cream on account of the mechanical losses incidental to the combustion of considerable amounts of fat, and the unavoidability of losses through spattering.
3. A method is proposed in which the residual solution from the official fat determination by the Roese-Gottlieb method is evaporated and ignited, and which gives reliable results.

REPORT ON FATS AND OILS.

By G. S. Jamieson (Bureau of Chemistry, Washington, D. C.), *Referee.*

During the past year study has been made of the Thomas and Yu[1] method for the separation and determination of peanut oil in admixture with other oils, and also of the André-Cook[2] method for the determination of acetyl value.

DETERMINATION OF PEANUT OIL.

The premise upon which the Thomas and Yu method exists consists of the differential solubilities of the magnesium soaps of the saturated and unsaturated acids occurring in peanut oil and the fractional crystallization of arachidic and lignoceric acids from other saturated acids. Advantage has been taken of the fact that magnesium soaps of higher saturated acids are practically insoluble in 90 per cent alcohol at 25°C. (0.007 gram of stearate and 0.006 gram of lignocerate per 100 cc.), while the oleate and other unsaturated acid soaps dissolve readily (8.60 grams of oleate per 100 cc. of 90 per cent alcohol). Considerable study of the method by the authors indicated that a clear-cut separation of peanut oil from olive and cottonseed oils could be readily obtained. Other oils, notably rape and tung oils, yielded insoluble magnesium soaps of unsaturated acids and precluded the use of the method for peanut oil in such admixtures.

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[1] *J. Am. Chem Soc.*, 1923, **45**, 113

[2] André. Compt rend , 1921, **172**. 984. See also *Bull. Soc. Chim* , (4), 1921, **29** 745 Cook. *J. Am. Chem. Soc.*, 1922, **44**: 392

The initial investigation carried out in the present study was a test on peanut oils of authentic origin. A cold-pressed oil from Spanish nuts and a hot-pressed oil from Virginia nuts were analyzed by the procedure recommended, and the results shown in Table 1 were obtained.

TABLE 1.

Results of analysis of peanut oils (Thomas and Yu method).

TYPE OF OIL	WEIGHT OF SAMPLE	WEIGHT OF ACIDS	WASHINGS 90% ALCOHOL AT 20°C.	CORRECTION	CORRECTED WEIGHT OF ACIDS	SATURATED ACIDS	PEANUT OIL INDICATED
	grams	*gram*	*cc.*	*gram*	*gram*	*per cent*	*grams*
Spanish	9.9819	0.4711	70	0.0609	0.5320	5.31	10.6400
	10.4050	0.5278	55	0.0500	0.5778	5.55	11.5560
	10.9898	0.5715	55	0.0528	0 6243	5.68	12 4860
Virginia	9.7327	0.4569	55	0.0478	0.5047	5.18	10.0940
	10.5491	0.4971	45	0.0409	0.5380	5.10	10.7600
	10 1329	0.4531	60	0.0522	0.5053	4.99	10.1060
	10.0826	0.4570	55	0.0478	0.5048	5.01	10.0960

The same oils were also analyzed by the Renard-Tolman[1] lead-salt-ether method, the results being shown in Table 2.

TABLE 2.

Results of analysis of peanut oils (Renard-Tolman method).

TYPE OF OIL	WEIGHT OF SAMPLE	WEIGHT OF ACIDS	VOLUME 90% ALCOHOL AT 20°C.	CORRECTION	CORRECTED WEIGHT OF ACIDS	SATURATED ACIDS	PEANUT OIL INDICATED
	grams	*grams*	*cc.*	*gram*	*grams*	*per cent*	*grams*
Spanish	21.6307	1.1337	110	0.0495	1.1832	5.47	23.664
	19.0650	0.9771	110	0.0495	1.0266	5.38	20.532
	19.5590	1.0282	110	0.0495	1.0777	5.51	21.554
Virginia	18.9600	0.8920	110	0.0495	0.9415	5.02	18.830
	22.5835	1.0729	110	0.0495	1.1224	4.97	22.448

After this preliminary treatment samples of peanut oil were mixed with a high-grade olive oil, and analyses were made of the mixtures so prepared. Oils No. 1 and No. 2 contained 25 per cent and 15 per cent of peanut oil, respectively, and oil No. 3 was a pure olive oil. These oils and directions for the separation were forwarded to collaborators, whose reports are mentioned later.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 296.

DETERMINATION OF PEANUT OIL IN OLIVE OIL.

Thomas and Yu Method.

(As submitted to collaborators.)

REAGENTS.

(a) *Alcohol solutions.*—Carefully prepare suitable quantities of 90 per cent and 70 per cent alcohol by volume. (This may be conveniently done by diluting 90 cc. of 95 per cent alcohol to 95 cc. with distilled water and 70 cc. of 95 per cent alcohol to 95 cc. with distilled water.)

(b) *Alcoholic acetic acid.*—Dissolve 40 cc. of glacial acetic acid in 160 cc. of 95 per cent alcohol.

(c) *Alcoholic magnesium acetate.*—Dissolve 50 grams of the pure salt in 100 cc. of water, heat to boiling, filter off any basic salt, and to the cool filtered solution add three volumes of 95 per cent alcohol.

(d) *Purified alcohol.*—Add 1.5 grams of silver nitrate dissolved in 3 cc of water to 1.1 liters of 95 per cent alcohol. Dissolve 3.0 grams of potassium hydroxide in 15 cc. of hot 95 per cent alcohol, cool, and add to the first solution. Shake, and allow to stand until the precipitate has completely settled, Decant or syphon off the solution for use.

(e) *Alcoholic potash.*—Dissolve 50 grams of pure potassium hydroxide in 1 liter of purified alcohol.

DETERMINATION.

Weigh accurately approximately 10 grams of the sample into an Erlenmeyer flask (300 cc.) and saponify it with a mixture of 50 cc. of alcoholic potash and 50 cc. of 95 per cent alcohol by heating to a gentle boil under a reflux condenser for 30 minutes. Neutralize to phenolphthalein, while still warm, with alcoholic acetic acid, finally adding a drop or two of alcoholic potash to give the solution a light but permanent pink color. Now add 25 cc. of magnesium acetate solution and heat to boiling. (Care must be taken here as the solution bumps violently owing to separation of magnesium soaps.) Allow to stand overnight at 10°C.

Filter the insoluble magnesium soaps upon an 11 cm. filter (a folded filter should not be used as it causes trouble in removing the soaps) and wash twice with 15 cc. portions of the 90 per cent alcohol previously used to rinse the precipitation flask. With a narrow spatula transfer as much as possible of the washed soaps to the original flask. Treat the residue upon the filter with 100 cc. of boiling 1 : 1 hydrochloric acid (to decompose the soaps), the filtrate being caught in the flask containing the bulk of the soaps. (Should all the acids fail to filter, the filter paper may be washed with two 15 cc. portions of boiling 90 per cent alcohol.) Boil the flask containing the soaps and acid for 5 minutes to complete the decomposition of the soaps, then cool rapidly in a stream of cold water while agitating vigorously, in order to separate the free fatty acids in small particles suitable for filtration and washing. Filter the free fatty acids upon an 11 cm. filter and wash free of magnesium chloride with distilled water. During the washing dry the precipitation flask at 100°–130°C. Transfer as much as possible of the free fatty acids from the filter to a 150 cc. beaker. Dissolve the portion remaining with three 20 cc. portions of the hot 90 per cent alcohol previously used to wash out the dried precipitation flask, catching the filtrate in the 150 cc. beaker containing the fatty acids. Heat to boiling and place when cool in a 20° or 25°C. thermostat and allow to stand overnight.

Filter the separated acids, and wash twice with 10 cc. portions of 90 per cent alcohol (20° or 25°C.). Collect and measure the filtrate and alcohol washings. Now wash

the acids on the filter with 70 per cent alcohol at 20°C. until 10 drops of the filtrate produce no turbidity with water. Dissolve the fatty acids upon the filter with boiling absolute alcohol, catching the filtrate in a weighed flask or beaker. Wash the original beaker thoroughly with absolute alcohol, pouring the washings through the filter to be sure all acids are dissolved. Evaporate the alcohol and dry the flask or beaker to constant weight. Correct this weight for arachidic and lignoceric acids dissolved in the 90 per cent alcohol filtrate and washings.

The mixed acids obtained should melt at from 71° to 73°C. Recrystallization from 90 per cent alcohol gives crystals that melt at 75°–76°C.

Multiply the corrected weight of the fatty acids by 20 to obtain the approximate quantity of peanut oil.

Solubility corrections for weighed acids per 100 cc. of alcoholic solution.

WEIGHT OF ACIDS	CORRECTIONS	
	At 20°C.	At 25°C.
gram		
0.60	0.099	0.172
0.55	0.096	0.167
0.50	0.091	0.162
0.45	0.087	0.157
0.40	0.083	0.152
0.35	0.079	0.147
0.30	0.075	0.142
0.25	0.070	0.137
0.20	0.066	0.132
0.15	0.062	0.127
0.10	0.059	0.122
0.05	0.054	0.117

COLLABORATIVE RESULTS AND COMMENTS.

Six sets of collaborative results were obtained on the mixtures of oils. These are given in Table 3.

In general it would seem that the results obtained are somewhat high, Oil 1 generally being reported about 10 per cent over that present and Oil 2 about 2 to 3 per cent too high. One analyst reported obtaining a residue of 0.0042 gram of fatty acid from Oil 3, a pure olive oil. It has been the experience of the writer that a weighable residue was obtained from the olive oil, and the reports of two collaborators indicate that considerable fatty acid was recovered by them. From these results it would appear that the magnesium soap method as now outlined allows the entrainment of some lower melting saturated acids, along with the arachidic and lignoceric acids, causing high results. It would seem that some slight change in the procedure might yield better results, and it is accordingly recommended that study of the Thomas and Yu method be continued.

ACETYL VALUE.

The past year's work, as recommended at the last annual meeting, was limited to a further study of the André-Cook method for the deter-

TABLE 3.

Collaborative results with Thomas and Yu method.

ANALYST	OIL NO. 1 25 PER CENT PEANUT OIL	OIL NO. 2 15 PER CENT PEANUT OIL	OIL NO 3 OLIVE OIL
	per cent	*per cent*	*per cent*
M. L. Sheeley	42.3	26.45	None
	42.7	26.95	None
W. D. Richardson			
Analyst No. 1	36.8	16.6	None
	34.0	17.3	None
Analyst No. 2	35.5	16.8	None
	35.0	14.5	None
C. Griggs	35.8	15.43	10.34
	35.8	15.49	9.50
	35.66	. .	10.15
A. Edeler	35.0	16.4	18.4
	34.4	16.7	17.1
	36.4	. .	
R. M. Hann	36.24	16.91	None
	36.84	17.33	None
	35.71	16.99	None

mination of the acetyl value. Since the previous collaborative study of this method failed to yield satisfactory results, a study was made at the Oil, Fat and Wax Laboratory to determine a more satisfactory procedure for the removal of the excess of acetic anhydride from the acetylated oil, because it was believed that the principal reason for the lack of agreement in the results obtained by different analysts was either the incomplete removal of the acetic acid or the partial hydrolysis of the acetylated oil owing to excessive washing with water. As a result of this investigation, detailed directions for the removal of the excess of acetic anhydride from the acetylated oil were included in the André-Cook method sent to the collaborators. The directions for the method were as follows:

ACETYL VALUE.

André-Cook Method.

Acetylation.—Boil 50 cc. of the sample with 50 cc. of freshly distilled acetic anhydride under a reflux condenser for 2 hours. Pour the mixture into 500 cc. of water in a beaker and boil for 15 minutes while bubbling a stream of air or carbon dioxide through the solution to prevent bumping. Syphon off the water, add 500 cc. more of water, and boil for 15 minutes. Syphon off the water and boil for 15 minutes with a third 500 cc. portion of water. Allow the mixture to cool and separate the water layer, which should be neutral to litmus. Transfer the acetylated oil to a separatory funnel and wash with two 200 cc. portions of warm water. Separate as much of the water as possible, add 5 grams of anhydrous sodium sulfate, and let stand an hour, agitating occasionally to assist in drying. Filter through a dry folded filter, preferably in an oven, and let the acetylated oil remain in the oven until it is completely dried. The acetylated product should be a clear brilliant oil.

Saponification.—Weigh accurately from 2 to 2.5 gram portions of the acetylated oil and the untreated oil into 250 cc. Erlenmeyer flasks. Add exactly 25 cc. of alcoholic potash (40 grams of potassium hydroxide per liter) to each flask. Heat the flasks with reflux condensers for 1 hour. Titrate the solutions with half-normal standard hydrochloric acid, using phenolphthalein as indicator. Also titrate two 25 cc. portions of the alcoholic potash solution with the standard acid solution. Deduct in each analysis the quantity of acid used from that required to neutralize the blank and calculate the saponification values of the oil and the acetylated oil. Calculate the acetyl value by the following formula:

$$A = \frac{S^1 - S}{1 - 0.00075\,S}, \text{ in which}$$

A is the acetyl value, S is the saponification value of oil, and S^1 is the saponification value of the acetylated oil.

COLLABORATIVE RESULTS AND COMMENTS.

Collaborators reported results that were in satisfactory agreement, showing that a standardized washing procedure undoubtedly aided in gaining more concurrent values. In this connection it may be pointed out that one drop of the standard hydrochloric acid solution used in titrating the saponified product may mean a difference of from 0.5 to 1.0 unit in the acetyl value. It follows that slight differences in the normality of the titrating acid of the various collaborators, ordinarily negligible, in this method may introduce appreciable variation in the acetylation value.

The collaborative results are summarized in Table 4.

TABLE 4.

Collaborative results with André-Cook method.

ANALYST	OIL A*	OIL B†	OIL C‡
	per cent	*per cent*	*per cent*
M. L. Sheeley	6.05	43.15	140.00
	6.06	43.60	140.40
C. Griggs	4.19	23.6	143.0
	5.04	21.9	144.1
A. Edeler	5.1	40.8	147.3
	6.7	41.6	148.2
W. D. Richardson	7.4	45.3	149.2
	8.3	44.9	149.1
R. M. Hann	7.5	44.2	149.3
	7.6	44.5	149.3
	7.9	44.1	149.1

* A Japanese rape seed oil.
† A mixture of 75 per cent rape and 25 per cent castor oil.
‡ Castor oil.

Sheeley writes: "The André-Cook method seems to give fairly satisfactory results and there seems to be no difficulty in obtaining checks".

Richardson comments as follows: "The work with the André-Cook method appeared to give quite satisfactory results, so far as check results were concerned, especially with the higher numbers".

Using a somewhat stronger alkali (50 grams of potassium hydroxide per liter), Edeler reports that he obtained somewhat higher values for Oil C, namely 149.9 and 149.4. These values are more closely in agreement with those of Richardson and Hann. With few exceptions the results are satisfactory, the mixed oil in particular yielding checking results, and it is accordingly recommended that the André-Cook method be adopted as an official method.

RECOMMENDATIONS[1].

It is recommended—

(1) That the F. A. C. method for the determination of unsaponifiable matter be made official (second reading).

(2) That the study of the Thomas and Yu method for the detection and determination of peanut oil alone or in the presence of other oils be continued.

(3) That the André-Cook method for the determination of acetylation value, as outlined in the report, be adopted as official (first reading).

(4) That a study be made of the determination of saturated and unsaturated acids by the lead-soap-ether and Twitchell's lead-soap-alcohol methods (new work).

REPORT ON BAKING POWDER.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Referee.*

The work on baking powder this year was largely a continuation of the work of last year. Collaborative studies were made on the determinations of carbon dioxide, lead, and the phosphates.

CARBON DIOXIDE.

The instructions sent to collaborators who had signified a willingness to make the determination of carbon dioxide were to use the reagents and apparatus as described in *Methods of Analysis, A. O. A. C., 1925,* page 305, but to make some modifications in the method. They were directed to use 1.7 grams of the sample instead of a factor weight as previously indicated and, after measuring the volume of gas evolved, refer to a table (furnished by the referee) for the factor to employ in calculating the percentage of carbon dioxide. This table, prepared by J. R. Chittick of Chicago, required a large amount of work, and the thanks of this association are due him for this contribution.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 72.

TABLE 1.

Carbon dioxide.

DATE	ANALYST	TOTAL CO_2	RESIDUAL CO_2	AVAILABLE CO_2
		per cent	*per cent*	*per cent*
5/10/26	G. A. McDonald Victor Chemical Works Chicago Heights, Ill.	13.8	0.8	13.0
5/11/26	M. R. Stanley Victor Chemical Works Chicago Heights, Ill.	13.9	0.7	13.2
5/12/26	A. H. Allen Virginia-Carolina Chemical Corp. Richmond, Va.	14.46	0.54	13.92
5/—/26	Grace Vincent Royal Baking Powder Co. Brooklyn, N. Y.	14.18	0.49	13.69
6/—/26	—— De Lemon Royal Baking Powder Co. Chicago, Ill.	14.19	0.49	13.70
-/—/26	J. T. Field* Food and Drug Inspection Lab. Minneapolis, Minn.	13.24	0.50	12.74
-/—/26	W. C. Luckow American Institute of Baking Chicago, Ill.	14.03	0.66	13.37
5/ 5/26	Milton H. Kemp Calumet Baking Powder Co. Chicago, Ill.	14.40	0.61	13.79
5/19/26	L. H. Bailey	14.03	0.49	13.54
-/—/—	Percy O'Meara Department of Agriculture Lansing, Mich.	14.20	0.41	13.79

* The apparatus used was a 100 cc. buret, instead of the regulation apparatus, which may account for the low results. These results are given by way of comparison, but they cannot be considered in connection with the other results.

Ten analysts collaborated in making determinations of carbon dioxide. One of the analysts did not have the apparatus specified in the method, and his results are about 1 per cent too low. They should not be considered with the others. Another analyst used a special gasometric apparatus, but as it is based on the same principles as the one described in the method his results are comparable. The other analysts secured concordant results. The averages of their several determinations are shown in Table 1. The agreement in results is not perfect, but it is fully as close as has been obtained by other methods. The referee recommends that the modified gasometric method as studied this year be made official because it is simple, rapid, and reasonably accurate.

TOTAL CARBON DIOXIDE.

PREPARATION OF SAMPLE.

Remove the entire sample from the package, pass through a 20-mesh sieve, and mix thoroughly.

REAGENTS.

(**a**) *Dilute sulfuric acid* (*1* + *5*).

(**b**) *Displacement solution.*—Dissolve 100 grams of sodium chloride or sodium sulfate crystals in 350 cc. of water. Add approximately 1 gram of sodium bicarbonate and 2 cc. of methyl orange indicator [p. 48, 3 (**f**)] and then sufficient of the dilute sulfuric acid (1 + 5) to make just acid (a decided pink color). Stir until all carbon dioxide is removed. This solution is used in the gas-measuring tube and leveling bulb and seldom needs to be replaced.

APPARATUS.

The apparatus, Fig. 21[1], consists of a decomposition flask (*A*) connected by means of a glass T-tube (*B*), provided with a stopcock (*C*), to a graduated gas-measuring tube (*D*), which in turn is connected with a leveling bulb (*E*). The decomposition flask consists of a 250 cc. wide-mouth extraction flask of Pyrex or other resistant glass fitted with a two-holed rubber stopper, through one hole of which passes the extended tip of a 25 cc. buret (*F*) and through the other a glass tube of the same diameter as the connecting T-tube. The 25 cc. buret is graduated in cc. at 20° C., numbered at 5 cc. intervals, and is provided with an extra long tip bent to pass through the rubber stopper. The glass tube leading from the decomposition flask to the T-tube is connected to the latter by means of rubber tubing to permit rotation of the flask. The gas-measuring tube is graduated in cc. at 20°C., the zero mark being placed at a point 25 cc. below the top marking to allow for graduating upwards from 0 to 25 cc. and downward from 0 to 200 cc. The gas-measuring tube is connected by means of a long rubber tube with the leveling bulb, which has a capacity of about 300 cc.

DETERMINATION.

Weigh 1.7 grams of the sample, prepared as above, into a dry decomposition flask and connect with the gasometric apparatus (Fig. 21). Open the T-tube stopcock and by means of the leveling bulb bring the displacement solution to the 10 cc. graduation above the zero mark. (This 10 cc. is equal in volume to the volume of acid to be used in the decomposition.) Allow the apparatus to stand 1–2 minutes to insure equalization of temperature and pressure within the apparatus with that of the room. Close the stopcock, lower the leveling bulb somewhat to reduce the pressure within the apparatus, and slowly run into the decomposition flask from the buret 10 cc. of the dilute sulfuric acid. To prevent the liberated carbon dioxide from escaping through the acid buret into the air, keep the displacement solution in the leveling bulb at all times during the decomposition at a lower level than that in the gas-measuring tube. Rotate and then vigorously agitate the decomposition flask to secure intimate mixture of the contents. Allow to stand for 5 minutes to secure equilibrium. Equalize the pressure in the measuring tube by means of the leveling bulb and read the volume of gas evolved. Read the temperature of the air surrounding the apparatus and also obtain the barometric pressure at the time the volume of gas is read. Refer to the table submitted[1] for value of carbon dioxide.

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[1] *Methods of Analysis*, *A. O. A. C*, 1925, 305.

RESIDUAL CARBON DIOXIDE.

Gasometric Method.

Weigh 1.7 grams of the sample, prepared as above, into the decomposition flask; add 20 cc. of water; and allow to stand for 20 minutes. Place the flask in a metal drying cell surrounded by boiling water and heat, with occasional shaking, for 20 minutes. To complete the reaction, heat quickly to boiling and boil for 1 minute. Cool to room temperature, connect the flask to the apparatus, and determine the carbon dioxide present by treating with 10 cc. of the dilute sulfuric acid. Proceed as directed for the determination of total carbon dioxide.

AVAILABLE CARBON DIOXIDE.

Subtract the residual carbon dioxide from the total carbon dioxide.

LEAD.

Under normal conditions lead in baking powder occurs in very small quantities. In fact, the quantities are so small that accurate determinations are difficult.

The electrolytic method now being studied by the association is the most promising of any method yet proposed. Heretofore the referee has had special baking powders prepared in which known quantities of lead salts were added. This year the collaborative work was done on commercial baking powder without the addition of any lead compound.

Very few laboratories are equipped with suitable electrical apparatus to make this determination, and accordingly only three collaborators were secured for this study. The results obtained by these collaborators are in good agreement, and the referee recommends that the electrolytic method for the determination of lead as published in *Methods of Analysis*, page 310, as a tentative method now be made an official method. Collaborative results are shown in Table 2.

TABLE 2.

ANALYST	LEAD	
	p.p.m.	
G. A. McDonald	4.5 5.0 4.5	4.7
Milton H. Kemp Calumet Baking Powder Co. Chicago, Ill.	2.56 3.85 3.20	3.2
James K. Morton Bureau of Standards Washington, D. C.	4.48	
Percy O'Meara	4.5 6.4 5.1	5.33

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[1] *This Journal*, 1927, **10**: 37. One error has been noted. In column 8, 15 lines from the bottom, change the factor 1.02212 to 1.02012.

PHOSPHATES.

The referee recommended last year that suitable methods be developed for the separation and estimation of meta-, pyro-, and orthophosphates in the presence of each other.

This task is not an easy one; several collaborators have been working earnestly with the problem, but they have not progressed sufficiently to make a final report. Some of them have made preliminary reports and asked for more time before submitting definite results. None submitted a complete method of separating and determining the three forms of phosphates. On the collaborative sample submitted for analysis, J. T. Field reported total phosphoric acid, P_2O_5, 49.06 and 49.12 per cent and pyrophosphoric acid (Fiske method), 24.56 and 24.60 per cent, and Milton H. Kemp reported 4.2, 4.2, 3.9, and 4.0 per cent, average of 4.07 per cent of metaphosphoric acid. His method follows:

Dissolve a 0.2 gram sample in 100 cc. of cold 5 per cent acetic acid. Filter the solution and wash with cold 5 per cent acetic acid. (This procedure should be carried out as quickly as possible.) Add 10 cc. of 10 per cent $BaCl_2$ solution to the filtrate. Allow the solution to stand overnight, filter the precipitate, wash with cold water, ignite, and weigh as $Ba(PO_3)_2$. This method is based upon the fact that $BaCl_2$ or $Ba(NO_3)_2$ will precipitate metaphosphoric acid but not ortho- or pyrophosphoric acids. The success of the method seems to depend largely upon how long the solution stands before precipitation. If it is allowed to stand too long some metaphosphoric acid will change over into orthophosphoric acid and thus lower the results.

RECOMMENDATIONS[1].

It is recommended—

(1) That the electrolytic method for the determination of lead, as published in *Methods of Analysis*, page 310, as a tentative method be made an official method (final reading).

(2) That the modified gasometric method for the determination of carbon dioxide, as studied this year, be made official (first reading).

(3) That study of methods for the separation and determination of ortho-, pyro-, and metaphosphates in the presence of one another be continued.

LITERATURE REFERENCES.

S. Aoyama. *Pharm. Soc.*, Japan, 1925, No. 520; 553-6. Volumetric Estimation of Ortho-, Pyro-, and Metaphosphoric Acids in Mixtures.

J. Tillmans and Anna Bohrmann. *Z. Nahr. Genussm.*, 1921, **41**: 1-17. Estimation of the Alkalinity and of Phosphates in the Ashes of Foods.

W. W. Scott. *Chem. News*, 1925, **131**: 17-20. Determination of Lead in Minute Quantity in Baking Powders, Lime, Alum, Tartrates, Citrates, and Carbonates in the Presence of Iron and Copper.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 73

DRUG SECTION.

REPORT ON DRUGS

By ARTHUR E. PAUL (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Referee.*

In harmony with the recommendations approved last year, twenty topics were selected for study, and the association appointed an associate referee for each of these topics. It was the hope of the referee that as a result of this year's work many of these subjects might be discontinued. In this hope, however, he was somewhat disappointed. Nevertheless, it is recommended that four topics be discontinued because the methods are deemed worthy of adoption. In addition, it is believed that the general status of the drug work has been materially advanced as a result of this year's work.

As in former years, the referee attempted to review all reports received. Many of these are splendid and represent definite progress. In a few instances urgent regular duties prevented the associates from performing any work. This condition is regretted, but it is, no doubt, unavoidable. The referee has made such comments in each instance as appeared necessary or desirable, in order to harmonize the reports with present methods and the usual procedure of this association.

The attached table will show in convenient form the present status of the subjects that were assigned this year:

Acetylsalicylic acid—No report received.

Alcohol in drugs—Continue topic. Study various alcohols in presence of each other and interfering substances.

Apomorphine—Adopt method and discontinue study.

Arsenicals—Adopt method and discontinue study.

Bioassay of drugs—Continue study.

Chaulmoogra oil—Continue study.

Chloroform and carbon tetrachloride—Adopt method. Study determination in mixtures.

Cocaine—Further study.

Crude drugs—No report received.

Ether—Further study.

Ipecac alkaloids—Further study.

Laxatives and bitter tonics—Continue study.

Mercurials—Adopt method. Continue study.

Microchemical methods for alkaloids—Continue study.

Nitroglycerin—Adopt methods. Close topic.

Pyramidon—Adopt amended method. Close topic.

Radioactivity in drugs and water—Adopt methods. Continue study.

Santonin—Report of associate referee not received.

Silver proteinates—Adopt method. Study alkalinity.

Terpin hydrate—Continue study.

GENERAL RECOMMENDATIONS.

It is recommended—

(1) That the following topics be discontinued:

Apomorphine,
Arsenicals,
Nitroglycerin, and
Pyramidon.

(2) That the following topics be continued:

Chloroform and Carbon Tetrachloride,
Cocaine,
Ether,
Ipecac Alkaloids,
Laxatives and Bitter Tonics,
Mercurials,
Microchemical Methods for Alkaloids,
Radioactivity in Drugs and Water,
Bioassay of Drugs,
Chaulmoogra Oil,
Silver Proteinates, and
Terpin Hydrate.

(3) That last year's recommendations respecting the following topics on which no reports were received from the respective associate referees be repeated:

Acetylsalicylic Acid,
Crude Drugs, and
Santonin.

The following comments are made by the referee for consideration in connection with the reports given by the various associate referees:

ALCOHOL IN DRUGS.

The associate referee last year studied methods for the determination of alcohol in the presence of a number of interfering substances. As very little collaborative work was reported, it was recommended that the methods, as revised by the referee, be resubmitted to collaborative study this year. However, no reports were received from collaborators, and no work was performed on the methods by the associate referee. Some collaborative work was done by C. K. Glycart, whose report, unfortunately, was made after the associate referee's report was mailed. This collaborator's report deals with samples prepared by himself, containing only two of the several interfering substances included in the associate referee's proposed methods. It seems, therefore, that the status of this work, so far as last year's recommendations are concerned, remains practically unaltered.

The associate referee this year suggests details for procedure in the presence of two additional interfering substances, acetaldehyde and methyl alcohol.

With respect to acetaldehyde or paraldehyde, both the associate referee and Glycart made some determinations. While the former reports satisfactory results, the latter offers some criticisms of the details and makes certain suggestions for clearness.

Relative to the proposed directions to be used in the presence of methyl alcohol, attention is called to the fact that the official methods already include three methods for the determination of methyl alcohol in the presence of ethyl alcohol[1], and that the method prepared by the associate referee constitutes an additional determination. It is believed that this method has distinct advantages over the two color methods given in *Methods of Analysis* (**16, 17**). It would seem desirable to correlate the methyl alcohol methods for drugs through a referee or associate referee, whose topic would include all methods for alcohol.

The associate referee also submits a suggestion made by one of the prospective collaborators to whom he sent his methods for study, H. Wales, that the determination of isopropyl alcohol be given consideration.

The associate referee makes no definite recommendations, but from his report and the consideration enumerated above, the following recommendations are now made by the referee:

RECOMMENDATIONS—ALCOHOL IN DRUGS.

(1) That the methods, as given in the referee's report last year, be submitted to collaborators for study.

(2) That to the methods so submitted, there be added the method proposed by this year's associate referee, but including the suggestions made by Glycart.

ARSENICALS.

Several methods for the determination of arsenic are now recognized in *Methods of Analysis* and in the U. S. Pharmacopeia. However, as pointed out by the associate referee, these are not satisfactorily applicable to iron-arsenic tablets. Because it is believed that the proposed method will serve a very useful purpose, in view of the satisfactory results reported by collaborators, it is considered that the associate referee's recommendation for tentative adoption might well be adopted.

COCAINE.

The associate referee states that owing to the instability of cocaine to the action of heat, acids, and alkalis, the usual methods are not applicable. In general, this is undoubtedly correct. In his method, therefore, he employs a procedure that has been tried for various alkaloid determinations. It includes extraction with petroleum ether from an alkaline solution, and then extraction of the ethereal solution with a measured volume of standard solution. Ordinarily, this procedure is considered less desirable than the usual alkaloidal method of extraction from an ammoniacal solution, evaporation, and titration of the alkaloids, although it may be necessary to resort to this in connection with cocaine. Attention is called to the fact that the American Drug Manufacturers Association has proposed a procedure which follows the ordinary alkaloidal method with certain precautions to be observed in the evaporation.

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[1] *Methods of Analysis*, *A. O. A. C.*, 1925, 372-3.

The volumetric method proposed by the associate referee for tentative adoption apparently yielded satisfactory results as reported by the collaborators.

The gravimetric check method results were somewhat less so, but the reports indicate that the method has its possibilities, particularly in view of the results obtained by the associate referee himself. As suggested, it seems probable that he employs certain details that are not described as clearly or definitely as may be desirable.

In view of the importance of this alkaloid, and because the volumetric method itself does not differentiate between cocaine and another alkaloid or mixture of alkaloids, the gravimetric check method would be very desirable. Possibly, volumetric details for the determination of the benzoic acid formed may be devised.

Since the associate referee's volumetric method yielded satisfactory results, it is considered desirable that it be adopted tentatively. It is also recommended, however, that before final adoption as official, this method and the additional details for the determination of benzoic acid be subjected to collaborative study in comparison with the method of the American Drug Manufacturers Association, which, amended, is as follows:

HYPODERMIC TABLETS—COCAINE HYDROCHLORIDE.

Count and weigh sufficient tablets corresponding to 2 grains of cocaine. Transfer to a small separatory funnel. Dissolve in the minimum amount of water. Make the solution slightly alkaline with ammonium hydroxide and shake out with several portions of ether until the aqueous layer is shown to be completely exhausted of alkaloid, using Mayer's reagent for the test. Combine the ether extracts and evaporate the major portion of the ether on the steam bath, finally allowing the remainder to be dissipated at room temperature. Dissolve the residue in a few cc. of neutral alcohol. Add 20 cc. of 0.05 *N* sulfuric acid, and titrate the excess of acid with 0.02 *N* sodium hydroxide, using methyl red indicator. (Each cubic centimeter of 0.05 *N* sulfuric acid corresponds to 0 0169̇83 gram of cocaine hydrochloride, $C_{17}H_{21}O_4NHCl$).

CHAULMOOGRA OIL.

It was recommended and approved last year that further study be given to color reactions of chaulmoogra oil and to loss or gain of weight on heating.

In the report this year, the associate referee states that no collaborative work was done.

In view of the fact that the recommendation for further work was approved, it is considered desirable to expedite the closing of this subject. It is suggested, therefore, that last year's recommendation be given appropriate attention next year.

CHLOROFORM AND CARBON TETRACHLORIDE.

The results reported by all collaborators are excellent. A number of these collaborators questioned the necessity for some of the steps required by the method. However, as the associate referee, by his own work, has shown the need for these details, it is thought desirable that the recommendation for the adoption of his method as tentative be approved. It is also suggested, in accord with his recommendation, that the details given for determining these constituents in complex mixtures be further studied next year.

IPECAC ALKALOIDS.

It seems from the associate referee's report that it was his intention to submit to collaborators two different samples of fluidextract, but that he failed to identify the two bottles that he submitted to them. Consequently, the two Chicago collaborators assumed that the two bottles were alike. As indicated in their communication, it was definitely shown that one of these contained approximately double the proportion of alkaloids. The third collaborator reported alkaloids approximately midway between the other two, showing that there was some confusion of the samples submitted.

While the work performed by this associate referee during several years has not yet resulted in a satisfactory conclusion, it is considered that material progress has been made in connection with this difficult determination.

The recommendations of the associate referee, that this topic be further studied with a view to the selection of the most serviceable procedure for tentative adoption by the association, are therefore approved.

RADIOACTIVITY IN DRUGS AND WATER.

In 1924 tentative methods for the determination of radioactivity were adopted. However, detailed methods for the preparation of diversified products preliminary to the actual determination were not included. The task of compiling such details was begun by the associate referee and his assistants two years ago because it was hardly possible, owing to the highly technical and specialized nature of this work, to conduct the investigation by means of the usual procedure, through collaborators.

Under the circumstances, it would seem desirable that the recommendations of the associate referee be approved, and that the details submitted by him be tentatively adopted. Also that this second recommendation, relative to next year's work, be given consideration.

In addition, it is recommended that the present tentative methods be now made official (first reading).

LAXATIVES AND BITTER TONICS.

No collaborative work was reported by the associate referee. However, a series of results on cascara bark and its fluidextract is given to show the values of the colorimetric determinations in comparison with the gravimetric results. The associate referee finds that the colorimetric results are approximate.

No methods have yet been adopted by the association, and no recommendation is made by the associate referee. In view of the fact that no collaborative work was done, it is recommended that study be continued as outlined in 1924 and approved last year.

MERCURIALS.

The recommendations of the associate referee are approved, and it is deemed desirable that the proposed method be adopted by the association as a tentative method.

PYRAMIDON.

Two tentative quantitative procedures are now available for the determination of this important synthetic drug, namely, an extraction method and a precipitation method. For the last two years, the associate referee has carefully studied, either personally or collaboratively, a modification of the extraction method that appears to have advantages. In view of the satisfactory results obtained it is suggested that the tentative method be amended according to the associate referee's recommendation, and that then this method as well as the precipitation method be adopted as official (first reading).

MICROCHEMICAL METHODS FOR ALKALOIDS.

While the microchemical examination of alkaloids is a rather old subject, so far as this association is concerned it is relatively new. However, it was believed that some of the available methods might be studied advantageously and later adopted as tentative or official.

At present a large number of alkaloids are known, but during the present year the associate referee confined his attention to five of the more important alkaloids. From the results reported by the collaborators, it seems that the methods submitted have merit and it is recommended that the study be continued next year.

SILVER PROTEINATES.

The following is quoted from a letter received from H. Wales:

> In glancing over the report "Changes made in the Official and Tentative Methods of Analysis, etc.", I note on p. 55 a method for the detection and estimation of ionizable silver compounds that has been made official (first action). In view of results obtained by a number of investigators in the last few years, I believe that this should read, "dialyzable silver compounds" and not "ionizable silver compounds". By means of potentiometer measurements several chemists have shown that in silver proteinates the percentage of silver more ionizable than AgI is considerably greater than that more ionizable than AgCl, i. e., furnishes a greater concentration of silver ion than AgCl. Also Smith and Giesy[1] found that for protargentum the dialyzable silver was approximately equal to that portion more ionizable than silver chloride. The question is—what is ionizable silver? In view of this it seems to me that the title of this subject should be changed to show exactly what is determined by the method.

The question raised by Wales is indeed interesting, but since it involves merely a change in designation, it is suggested that the matter be referred for consideration to the Committee on Editing Methods of Analysis.

The associate referee has called attention to a lack of clearness and definiteness in the description of the present official method and makes a suggestion as to an amendment that would provide the desired clearness. It is believed that the three recommendations made by him are desirable and that they should be approved by the association.

It is suggested that this topic be considered closed with the exception that, as recommended last year, consideration be given to the determination of alkalinity or acidity in this class of compound.

NITROGLYCERIN.

The associate referee's attention has been confined to the determination of nitroglycerin in tablets. The method that was studied last year and again this year is highly satisfactory, and the abbreviated procedure that was also studied this year is likewise desirable. In accord with the associate referee's suggestion, it is now recommended—

(1) That the modified Devarda method, described as Method No. 2, on page 319, Vol. IX, *This Journal,* be adopted as a tentative method for the determination of nitroglycerin in tablets.

(2) That the abbreviated details, described by the associate referee and referred to by him as "C", be adopted as a tentative method for the determination of nitroglycerin in tablets.

TERPIN HYDRATE.

As pointed out by the associate referee, the determination of terpin hydrate is a decidedly difficult problem, one that he was unable to solve satisfactorily owing to the limited time. In view of the importance of the topic and the absence of any usable methods, it seems desirable that the work be continued.

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[1] *J. Am. Pharm. Assoc.*, 1925, **14**: 10.

APOMORPHINE.

A method for the determination of apomorphine was studied last year by Associate Referee Glycart, and a report was made[1]. It may be stated that the method studied last year corresponds closely to Method I in the present report.

The results reported last year were fairly satisfactory. However, the associate referee did not recommend adoption, but rather pointed out some details for study. The points raised are met, at least in part, in this year's report, and the results reported by both methods are very acceptable.

The referee approves of this method being adopted as tentative and suggests that the topic be considered closed.

ETHER.

It will be noted that the associate referee scanned the literature on the subject of ether, and has decided upon a line of investigation that appears to him to have possibilities.

It is recommended, therefore, that this subject be continued along the lines indicated by him.

BIOASSAY OF DRUGS.

The associate referee reports on the bioassay of mydriatics, which consists of a quantitative determination for atropine hyoscyamine and scopolamine (hyoscine) in drug samples by the cat's threshold method. The assay was developed primarily for use on mixtures such as morphine and atropine in tablets when chemical methods are not suited or adequate.

Although certain details concerning the samples submitted for collaborative study are omitted in the report, the comments of the collaborators show that the method is satisfactory.

Therefore, the recommendations of the associate referee that these methods be adopted as tentative are approved by the referee.

No report on acetylsalicylic acid was given by the associate referee.[2]

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[1] *This Journal*, 1926, **9** 323.

[2] For report of Sub-committee B and action of the association, see *This Journal*, 1927, **10** 67.

REPORT ON ALCOHOL IN DRUGS.

By E. V. LYNN[1] (Food and Drug Inspection Station, Seattle, Wash.), *Associate Referee.*

The associate referee continued the study of interfering substances and of the estimation of small quantities of alcohol, as recommended last year[2].

It was found that aldehyde or paraldehyde can be effectively removed by alkaline silver nitrate solution without in any way interfering with the determination of alcohol. Experiments illustrative of the results obtainable are given as follows:

	I	II	III	IV
Volume of absolute alcohol in 100 cc.	9.27	....	....	....
Percentage of aldehyde or paraldehyde added	3.00	2.00	2.00	2.00
Percentage of alcohol recovered	9.25	9.27	9.25	9.27

If methyl alcohol is present in the sample, ascertain its percentage by a colorimetric determination and subtract the result from total alcohol, as indicated by specific gravity. The gravity of both alcohols is so nearly identical that no appreciable error is thus introduced. Some of the results obtained are shown:

	STANDARD	STANDARD DILUTION	I	II	III
Percentage of methyl alcohol	1.00	0.002	1.00	1.00	1.00
Percentage of ethyl alcohol	10.00	.....	9.57	9.57	9.57
Reading (Duboscq)		50	47	52	53
Percentage of methyl calculated			1.05	0.96	0.95
Percentage of ethyl calculated			9.52	9.61	9.62

It is recommended, therefore, that the following paragraphs be added to the report of the referee as given last year under the heading "Preparation of Sample":

In the presence of acetaldehyde or paraldehyde, add an excess of Tollen's reagent (alkaline silver nitrate solution)[3] and heat on the water bath under a reflux condenser for 30 minutes. Cool, and proceed as directed.

In the presence of methyl alcohol, prepare a standard solution containing 0.1 per cent of methyl and 0.9 per cent of ethyl alcohol. Dilute the distillate from the determination below so that it contains about 1 per cent of the mixed alcohols, noting the amount of dilution. To 2 cc. each of this dilution and the standard solution, add 2 cc. of 3 per cent potassium permanganate solution and 0.2 cc. of concentrated sulfuric acid. After 5 minutes, decolorize with sulfurous acid, add 1 cc. of concentrated sulfuric acid and 5 cc. of fuchsin-sulfurous acid T. S. (U. S. P., page 488), and dilute each in a standard colorimeter until the colors are equal. From the results, calculate the percentage of methyl alcohol in the sample.

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[1] Presented by V. K. Chesnut.

[2] *This Journal*, 1926, 9: 282.

[3] Prepare a solution containing 10 per cent silver nitrate in a mixture of equal parts of water and strong ammonium hydroxide (sp gr 0.90). To prepare the reagent mix equal parts of the ammoniacal silver nitrate solution and 10 per cent of aqueous sodium hydroxide solution. Keep the solution of silver nitrate in diluted ammonium hydroxide in stock but do not mix with sodium hydroxide until needed because on long standing the mixture deposits a highly explosive black precipitate.

In the event that the quantity of alcohol in the sample is very small, it has been shown that it is only necessary to redistil into 50 cc. as shown by the following results:

	per cent	*per cent*	*per cent*	*per cent*
Alcohol	2.00	0.95	0.49	0.25
First distillate	1.96	1.10	0.44	0.33
Second distillate	2.00	0.96	0.48	0.24

RECOMMENDATION[1].

It is recommended that the following paragraph be added to the method under "Determination" as recommended last year:

If the sample contains a very small quantity of alcohol, redistil the distillate into a volume of 50 cc.

REPORT ON ARSENICALS.

By H. WALES (Drug Control Laboratory, Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The official methods for the determination of arsenic are not applicable to iron-arsenic tablets. A few methods have been given in the literature, but none compares in simplicity and accuracy with that developed by A. Hansen[2]. This method is a modification of the Ramberg-Sjöström method[3]. It was awarded a prize given by the Danmarks Apotekerforenings for a method which, besides other requirements, should use not more tham 10 pills, each pill to contain from 0.5-1.0 mg. of arsenic trioxide.

It is of interest to note that the author recommends ferrous sulfate for reduction in place of the expensive hydrazine sulfate, which is apparently contradictory to the A. O. A. C. findings. This is not contradictory, however, for two reasons: first, all nitrates are destroyed before distillation; and second, it is claimed that the reduction in this case as as well as with hydrazine sulfate is in reality accomplished by the bromide in accordance with the following equations:

$$As_2O_5 + 4HBr = As_2O_3 + 2Br_2 + 2H_2O,$$
$$As_2O_3 + 6HCl = 2AsCl_3 + 3H_2O,$$
$$2FeSO_4 + Br_2 + H_2SO_4 = Fe_2(SO_4)_3 + 2HBr, \text{ or}$$
$$H_2NNH_2 + 2Br_2 = N_2 + 4HBr.$$

The first reaction is believed to take place in the cold when the sulfuric acid content is over 26 per cent, but this has not been proved.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**: 67.
[2] *Archiv. Pharm. Chemi*, 1925, **32**, Nr. 14-15: 208; *Pharm. Zentr.*, 1925, **66**: 701
[3] Arsenikkommission. Bil. VII-X. Lund, 1919; *Analyst*, 1925, **50** 6

Hansen claims that results of equal accuracy were obtained with either of the reducing agents. An investigation of this data shows that eight determinations in which ferrous sulfate was used gave an average variation of ± 0.018 per cent, while the same number with hydrazine gave an average of ± 0.025 per cent. The author claims an accuracy of between 0.125 and 0.25 per cent.

Hansen used the Ramberg-Sjöström arsenic flask for the determinations. This consists of a 300 cc. Kjeldahl flask with the outlet tube ground into the neck. Excellent results have been obtained in this laboratory by using an outlet tube of about 15 mm. diameter fastened to a Kjeldahl flask through a rubber stopper, the lower end of the tube being drawn out to about 5 mm. diameter.

This method, as reported by the associate referee, has been published[1].

COLLABORATIVE WORK.

Two samples of pulverized tablets, together with their average weights, were sent to the collaborators. No. 1 consisted of compressed tablets of arsenic trioxide, labeled 1/30 grain, and No. 2, chocolate coated Blaud and arsenic tablets, labeled 1/40 grain arsenic trioxide.

Collaborators outside the Government laboratories were secured through the courtesy of the chairmen of the contact committees of the American Drug Manufacturers Association and the American Pharmaceutical Manufacturing Association.

COMMENTS OF COLLABORATORS.

Robert McNeil, Philadelphia, Pa.: Preliminary experiments did not work out satisfactorily. We have been unable to complete the tests. I doubt very much whether it would ever prove to be a very popular method in pharmaceutical laboratories. The large amount of evaporation of strong acid is rather an objectional feature.

The Zemmer Co.: With the coated tablets, the addition of ferrous sulfate always produced an evolution of free bromine and, so far, we have had no success with the coated tablet.

We found it necessary to add ammonium oxalate at least two and sometimes three times in order to get the nitric acid entirely out of the solution and, also, the solution had to be heated 20 to 30 minutes after the evolution of sulfur trioxide. With a standardized solution of arsenic trioxide alone we were able to get practically 100 per cent results. The method appears to be rather long to be used in control work in a manufacturing plant.

Parke, Davis & Co.: To get good results with the method required some experience, our first experiments being rather erratic. After the analyst had acquired a little experience following the method in detail it appeared to give good results * * *. The method is apparently accurate, but it is almost too lengthy to be used as a rapid check assay in the course of manufacture.

C. K. Glycart: The method requires practice, especially experience with the titration. This, no doubt, is the reason for the lack of agreement in results on Sample No. 1.

E. O. Eaton: I had some difficulty with Sample No. 2. Apparently I had not removed

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[1] *This Journal*, 1927, **10**. 44.

TABLE 1.

Collaborative results on determination of arsenic in pills.

COLLABORATOR	A. O. A. C SAMPLES		BLAUD AND KNOWN As_2O_3		KNOWN As_2O_3	
	No. 1	No. 2	Taken	Found	Taken	Found
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
A. G. Murray Bureau of Chemistry Washington, D. C.	1.965 2.065 1.98 1.99 1.96	1.59 1.605	18.0 20.0 22.0	18.1 20.06 21.98	20.01 20.01	20.11 20.06
E. O. Eaton U. S. Food and Drug Inspection Station, San Francisco, Calif.	1.97	1.57				
C. K. Glycart U. S. Food and Drug Inspection Station, Chicago, Ill.	2.03 2.11	1.62 1.60				
E. L. Anderson U. S. Food and Drug Inspection Station, Baltimore, Md.	1.95 1.93 1.94	1.54 1.55 1.52				
H. R. Smith U. S. Food and Drug Inspection Station, Baltimore, Md.	1.94 1.94	1.56 1.57				
H. Wales	1.99 1.99 1.99	1.64 1.60 1.57 1.60	10.44 10.44	10.42 10.45		
Powers, Weightman, Rosengarten Co., Philadelphia, Pa.	1.97 1.90 1.91	1.60 1.49 1.49	2.64* 2.64	2.71* 2.67	See No.	table 2
Norwich Pharmacal Co. Norwich, N. Y.	2.07 2.16 2.025	1.59 1.605				
Parke, Davis & Co. Detroit, Mich.	2.0 1.995 2.0	1.60 1.55 1.60				
The Upjohn Co. Kalamazoo, Mich.	1.92 1.91	1.55 1.56				
The Zemmer Co. Pittsburgh, Pa.	2.08 2.02					

* **Results expressed in percentage.**

all the nitrogen compounds because I noted a fading of the methyl orange without the addition of the bromate solution. Further work seemed to overcome this difficulty, and I have obtained the results as reported.

The Norwich Pharmacal Company: The method is good, and in the hands of an experienced operator it is undoubtedly highly accurate * * *. The blank upon the reagents used was less than 0.05 cc.

Powers, Weightman, Rosengarten Co.: We have had two of our chemists make these tests. One of them, W, used a bromate solution, 1 cc. of which corresponded to 2 mg. of arsenic trioxide. The other, F, used a solution, 1 cc. of which corresponded to 1 mg. of arsenic trioxide.

We also made a few assays by your method, but in place of bromate we titrated with 0.05 *N* iodine in the presence of an excess of bicarbonate and used starch indicator.

Our blanks, using U. S. P. ferrous sulfate, amounted to 0.2 cc. of the stronger bromate solution or 0.4 cc. of the weaker. When a higher grade of ferrous sulfate was used there was practically no blank. Chemist W checked his own results very closely. Chemist F made the assays only once.

With the bromate titration at room temperature the end point is rather slow. It is our opinion that heating the solution to 80°–90°C. before titration should be definitely recommended * * *. We prefer the iodine titration because it can be done readily in the cold and because the end point is more striking.

TABLE 2.

Comparisons of bromate and iodine titrations.

(Results from laboratory of Powers, Weightman, Rosengarten Co.)

SAMPLE MARKED	ANALYST	BROMATE TITRATION		IODINE TITRATION	
		As_2O_3 per tablet		As_2O_3 per tablet	
		per cent	*mg.*	*per cent*	*mg.*
1–1926 Weight of tablet: 47.156 mg.	W	4.18	1.97	4.05	1.91
	F	4.02	1.90		
2–1926 Weight of tablet: 533.32 mg.	W	0.30	1.60	0.28	1.49
	F	0.28	1.49		
Laboratory preparation Theory, 2.64 per cent	W	2.71		2.67	

U. S. Food and Drug Inspection Station, Baltimore, Md.: The results obtained by two analysts check very closely. After considerable experimenting and working with this method, the analysts have been able to get good results on the determination of arsenic in a number of different products.

Our chief trouble at first was due to liberation of hydrogen sulfide, which distilled over with the arsenic chloride. We believe that this hydrogen sulfide was derived from the rubber stoppers, because after boiling these stoppers with strong hydrochloric acid and thoroughly washing them, no further evolution was detected.

The method is easy to manipulate, and the results were quickly obtained. We do not find it necessary to cool the flask in which the distillate is received. The end point is made considerably sharper if the solution is heated to 90°C. before titrating with standard bromate solution.

H. R. Smith made a study of this method before adopting it for the use of the food chemists in the determination of arsenic in fruits which have been contaminated with arsenical sprays, and his results have shown it to be quite sensitive and accurate.

We believe that the uses for this method could be extended, and its general utility thereby increased.

DISCUSSION.

In addition to the applicability of this method for quantities of arsenic too large for the Gutzeit method and too small for precipitation methods, it also seems adaptable for the determination of arsenic in any quantity and in any form, except as cacodylates and methyl arsenates, which are not always decomposed by this treatment. These compounds should be heated with sulfuric acid, potassium sulfate, and starch, as described by C. K. Glycart[1], for destruction of the organic material, after which the arsenic can be distilled as previously described. Any adverse criticisms of the method are based on its length and the fact that some experience with it is necessary before accurate results can be obtained. Those that have done much work with the method praise it, and the results reported by the collaborators are in excellent agreement.

As originally sent to the collaborators, the method provided for titration at room temperature or at 90°C. It is believed that the higher temperature should be eliminated, since several have reported trouble due to nitrous fumes. Also, it has been thought advisable to give iodine as an alternative titrating agent, because the A. O. A. C. methods for the determination of arsenic in insecticides by distillation provide for titration by either standard bromate or iodine solution.

RECOMMENDATION[2].

In view of the very satisfactory results obtained by the collaborators, it is respectfully recommended that the method presented for the determination of arsenic in iron-arsenic tablets be adopted as a tentative method.

REPORT ON COCAINE.

By ELGAR O. EATON[3] (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Associate Referee.*

No attempt will be made to review the literature on the subject of cocaine as it is too voluminous. Much work has been done, and many methods have been recorded. For analytical purposes the use of an optical method does not appear feasible, but its use to check identity is valuable. Due to the instability of cocaine when subjected to heat or to the action of acids and alkalis the usual alkaloidal methods are not applicable. The easy hydrolysis of cocaine into definite end-products is taken advantage of in the methods described in this report, and the procedure is designed to overcome the objections ascribed to the usual methods. In these methods it is assumed that cocaine is the only alkaloid present, and if it is not, suitable means should be employed for its separation. This, however, is not always possible. Therefore, the benzoic acid determination herein described will prove of value as a check in conjunction with the titration. Specific identification tests as outlined by Warren[1] should be used in doubtful cases.

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[1] *This Journal*, 1926, 9. 286.

[2] For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9 67.

[3] Presented by A. G Murray.

METHODS[2].

Volumetric.

Take a sufficient quantity of a uniform sample to represent approximately 0.2 gram of the alkaloid. Dissolve in 20 cc. of cold distilled water and add 2 drops of dilute hydrochloric acid. Transfer to a separatory funnel. Make alkaline, to litmus, with a freshly prepared saturated solution of sodium bicarbonate. Shake out to exhaustion with petroleum ether. (Four 20 cc. portions are usually sufficient.) Combine and filter through a paper moistened with petroleum ether and wash the filter with petroleum ether. Transfer to a separatory funnel and add a decided excess of 0.02 N sulfuric acid and shake violently. Separate and wash with two 10 cc. portions of water. Combine the acid washings. Titrate back with 0.02 N alkali, using methyl red as the indicator. Calculate to cocaine hydrochloride. Cc. 0.02 N acid × 0.006793 = grams of cocaine hydrochloride in sample taken.

Gravimetric.

Add 10 cc. of 2.5 N sodium hydroxide solution to the titrated alkaloidal solution and evaporate to about 10 cc. on the steam bath. Cool, transfer to a separatory funnel, make acid with dilute hydrochloric acid, and exhaust with chloroform. Combine chloroform and wash with 5 cc. of water. Run chloroform through a plug of cotton previously moistened with chloroform. Evaporate spontaneously in a tared beaker. Dry in a vacuum desiccator for 2 hours and weigh[3]. Calculate benzoic acid weighed to cocaine hydrochloride. Weight found × 2.782 = grams of cocaine hydrochloride in sample taken.

COLLABORATIVE WORK.

A uniformly powdered mixture of lactose and 20 per cent by weight of cocaine hydrochloride was submitted to collaborators, whose reports are given in the table on p. 349.

The collaborators commented in part as follows:

H. Wales: Apparently either the temperature or alkali concentration is not correct for complete hydrolysis * * *.

C. W. Harrison: I have never considered the use of standard acid as a medium in shaking out a solution as is directed in the method a sound procedure. It seems to me for many reasons that this is a dangerous procedure and very liable to lead to poor results * * *.

L. E. Warren: Did not consider it necessary to shake out the titrated alkaloid before hydrolysis. (This procedure was recommended in the method as originally sent out.)

A. W. Hanson: The liberation of benzoic acid is a valuable confirmatory test for cocaine. It is possible that some of the cocaine was decomposed in the titration and not recovered in the ammoniacal shake-out. I would suggest that the solution after titration be evaporated to a small volume, hydrolyzed with sodium hydroxide, acidified with dilute hydrochloric acid, and extracted with chloroform. (This step was included in the second part of the method and reports submitted herewith are on the method as outlined above.)

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[1] *J. Am. Pharm. Assoc.*, 1923, **12**: 512.
[2] *This Journal*, 1925, **8**: 572.
[3] Clark, A. H. *J. Am. Pharm. Assoc.*, 1926, **15**. 6.

COLLABORATOR	VOLUMETRIC		GRAVIMETRIC
	per cent	*per cent*	*per cent*
H. Wales	20.2	19.8	16.0
Bureau of Chemistry	20.6	19.9	17.7
Washington, D. C.	19.9		16.9
	19.8		
	19.9		
C. W. Harrison	19.4		19.0
U. S. Food and Drug Inspection Station	19.2		
Baltimore, Md.			
L. E. Warren	19.2	21.0	20.0
Bureau of Chemistry	19.5	20.3	18.6
Washington, D. C.	19.6	20.0	19.0
	19.9	19.8	18.2
	20.4		18.9
A. W. Hanson	19.3		19.1
U. S. Food and Drug Inspection Station	19.0		18.7
Chicago, Ill.	19.5		18.5
	19.3		
	19.2		
E. O. Eaton	19.6		19.2
	19.6		19.3
	19.5		19.6
			19.5
Average recovery	98.5		93.0

E. O. Eaton: I found practically the same results by the gravimetric method in original and revised procedures. Evidently some detail in the procedure is obscure. A method for the quantitative determination of cocaine should be based on its hydrolytic end-products.

CONCLUSIONS.

The volumetric method appears satisfactory.

The gravimetric method is not so satisfactory, but it is, in conjunction with qualitative tests, an indication of the presence of cocaine.

RECOMMENDATIONS[1].

It is recommended—

(1) That the volumetric method be adopted as tentative.

(2) That the determination of benzoic acid from hydrolyzed cocaine be further studied.

REPORT ON CHAULMOOGRA OIL.

By L. E. WARREN (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

No collaborative work on chaulmoogra oil was carried out this year, the chief reason being lack of time on the part of the associate referee, but there were other deterring factors.

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[1] For report of Sub-committee B and action of the association, see *This Journal,* 1926, **10**: 68.

This oil is now described in United States Pharmacopeia X. As pointed out in previous reports by the associate referee[1], the tests in U. S. P. X are adequate for determining the identity and purity of chaulmoogra oil with the exception of possible adulteration with castor oil. The alcohol solubility test adopted as a tentative method last year by the A. O. A. C. excludes such adulteration within narrow limits. Two importers of chaulmoogra oil who collaborated last year no longer handle the product, so that it did not seem best to ask them to do collaborative work again. However, some progress was made with the bibliography, which is now chiefly therapeutic. In general, the clinical reports continue to be favorable. Some relapses have occurred in cases that appeared to be clinically cured, but this was to be expected. Some advanced cases have proved refractory to treatment. Improvement results, however, in most cases if the treatment can be continued.

Efforts are being made the world over to find new and possibly cheaper sources of oils that may be used in place of chaulmoogra oil. An oil from a Brazilian tree *Carpotroche brasiliensis*[2] has recently been brought to the attention of leprologists as possessing marked therapeutic properties in the treatment of leprosy. The oil has been examined by André and Moureu[3] and found to possess chemical and physical properties similar to those of chaulmoogra and hydnocarpus oils. These chem-

TABLE 1.

Comparison of carpotrochea oil with several oils of the chaulmoogra group.

KIND OF OIL	SPECIFIC GRAVITY	INDEX OF REFRACTION	OPTICAL ROTATORY POWER	SAPONIFICATION NUMBER	IODINE ABSORPTION NUMBER	MELTING POINT
Carpotroche brasiliensis	0.9499 (at 32°C.)	1.4755 (at 31°C.)	+53.67°	183.7	106.1	21°–23°
Taraktogenos Kurzii	0.9425 (at 32°C.)	. . .	48.0°	210.4	96.1	33°–39°
Hydnocarpus anthelmintica	0.9427 (at 32°C.)	1.4742 (at 29°C.)	48.0°	187.3	88.3	25°–26°
	0.9447 (at 29°C.)	1.4755 (at 29°C.)	58.17°	191.0	90.0	26°–29°
Hydnocarpus alpina	0.9346 (at 32°C.)	1.4764 (at 29°C.)	57.00°	201.0	95.0	20.5°
Hydnocarpus wightiana	0.9330 (at 32°C.)	1.4780 (at 29°C.)	61.67°	197.2	103.0	28°–32°
Asteriastigma macrocarpa	0.9217 (at 32°C.)	1.4725 (at 25°C.)	44.0°	189.4	82.8	37°–39°
Oncoba echinata	0.9286 (at 32°C.)	1.4740 (at 31°C.)	56.17°	184.5	98.0	40.5°–41.5°

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[1] *This Journal*, 1925, **8**: 515; 1926, **9**: 290.
[2] Machado. *J. Am. Med. Assoc.*, 1926, **87**: 711.
[3] *Compt. rend.*, 1925, **181**: 1089.

ists compared carpotrochea oil with several other oils of the chaulmoogra group. Their findings are given in Table 1.

Steps are now being taken by the Department of Agriculture to secure a specimen of carpotrochea oil for study.

No report on crude drugs was made by the associate referee.

REPORT ON CHLOROFORM AND CARBON TETRACHLORIDE.

By H. O. MORAW[1] (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee.*

In accordance with the recommendations adopted by the association last year, the method employing saponification under pressure was further studied by the following collaborators on known samples:

Valaer, Blaisdell, and Bradshaw, Bureau of Internal Revenue, Washington, D. C
P. W. Morgan, U. S. Food and Drug Inspection Station, Chicago, Ill.
Elemer Schulek, Budapest, Hungary.
H. Wales, U. S. Bureau of Chemistry, Washington, D. C.
C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.
L. E. Warren, Bureau of Chemistry, Washington, D. C.
H. O. Moraw.

Preliminary study on the quantitative separation of chloroform in mixtures was conducted by the associate referee, the proposed method for the determination being used. This study included the following mixtures:

1. White Pine Bark Cough Sirup, to which was added known quantities of chloroform. This mixture was distilled, and the distillate was passed into the reagent in the pressure bottle, which was surrounded by ice.
2. Water plus a known quantity of chloroform distilled into ice cold water, the reagent being added after distillation was complete.
3. Equal parts of alcohol and water plus known quantities of chloroform. The mixture was distilled, and the distillate was passed into the alcoholic potash reagent contained in the pressure bottle, which was cooled by ice.
4. Equal parts of alcohol and water distilled into the reagent, a known quantity of chloroform being added to the mixed reagent and distillate.

The results of this work are given in Table 1.

The associate referee also made a large number of determinations to secure data bearing on the following uncertain points:

(1) Whether heating is necessary to complete the reaction.

(2) Effect of varying head space in the pressure bottles.

The results of these determinations are given in Table 2.

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[1] Present address: Swan-Myers Co., Indianapolis, Ind. Report presented by L. E. Warren.

TABLE 1.

Results of separation and determination of chloroform in mixtures.

DESCRIPTION OF SAMPLE	CHLOROFORM PRESENT OR ADDED	CHLOROFORM FOUND	RECOVERY	DISTILLATE RECEIVED IN—
	gram	*gram*	*per cent*	
Sirup of White Pine Bark	0.1893 0.1717	0.177 0.1586	93.6 92.3	Usual reagent in pressure bottle cooled by ice
Sirup of White Pine Bark	0.1933 0.1658	0.1715 0.1536	88.7 92.7	Same as above. Stoppers completely protected by tinfoil
Distilled water	0.2237 0.1695	0.1044 0.1245	46.9 73.8	Water cooled by ice. Reagent added afterward
Equal parts of alcohol and water	0.1731 0.2164	0.1657 0.2058	95.75 95.1	Usual reagent cooled by ice
Equal parts of alcohol and water—$CHCl_3$ not distilled. Added to distillate and reagent*	0.1582	0.1580	99.8	$CHCl_3$ added to reagent and distillate but not distilled

* To determine the effect of dilution of reagent by the alcohol and water distilled as in the usual procedure.

Regular pressure bottles, Eimer and Amend, No. 1064, with rubber gaskets of 60 to 70 cc. capacity, were sent to all collaborators.

PREPARATION AND TESTS OF PURITY OF COLLABORATIVE SAMPLES.

Chloroform Sample No. 1.

Prepared by shaking occasionally during 24 hours 800 grams of U. S. P. chloroform, protected from light, with 160 grams of sulfuric acid. The separated chloroform was shaken with 40 grams of dried sodium carbonate for 30 minutes. The filtered chloroform was distilled at 60° to 61°C. into a cooled brown bottle to a volume of 510 cc. To this was added 3.78 grams of absolute alcohol. The following tests were made on the sample:

TESTS ON COLLABORATIVE SAMPLE NO. 1—CHLOROFORM.

Specific gravity	See Table 1
Non-volatile residue at 100°C. (U. S. P. limits 0.001g./50 cc.)	0.0000
Foreign odor, U. S. P. test	None
Chlorides, free chlorine, U. S. P. tests	None
Substances decomposable by sulfuric acid	None
Odorous or chlorinated decomposition products	None

Collaborative Sample No. 2

Collaborative Sample No. 2 consisted of repurified carbon tetrachloride without added preservative, prepared by treating highest purity carbon

TABLE 2.

Effect of saponifying chloroform and carbon tetrachloride under pressure with varying head space and with and without heat.

KIND OF BOTTLE	SIZE	HEAD SPACE	TREATMENT	USED	FOUND		REMARKS
				gram	*gram*	*per cent*	
CHLOROFORM:							
Regular pressure	60–70 cc.	None	Stood overnight unheated	0.1660 0.2515	0.1610 0.2442	96.98 97.10	Filled with C_2H_5OH
Magnesium citrate	12 fl. ozs.	11 fl. ozs.	Stood overnight unheated	0.2444 0.1530	0.2382 0.1512	97.5 98 8	
Magnesium citrate	12 fl. ozs.	11 fl. ozs.	Heated 3 hrs. at 98°C.	0.2312 0.2505	0.2321 0.2508	100.3 100.1	
Regular pressure	60–70 cc.	None	Heated 3 hrs. at 85–90°C.	0.1741 0.1544	0.1742 0.1546	100.0 100.1	Filled with C_2H_5OH
Regular pressure	60–70 cc.	30 cc.	Heated 3 hrs. at 98°C.	0.2880	0.2884	100.1	
Regular pressure	60–70 cc.	None	Stood over 2 days unheated	0.2146 0.1997	0.2104 0.1965	98.0 98.4	Filled with reagent
CARBON TETRACHLORIDE:							
Magnesium citrate	12 fl. ozs.	11 fl. ozs.	Stood overnight unheated	0.2535 0.2521	0.1107 0.1541	43.7 61.1	25 cc. C_2H_5OH added
Regular pressure	60–70 cc.	None	Stood overnight unheated	0.2291 0.2184	0.0148 0.1030	6.5 47.1	
Regular pressure	60–70 cc.	30 cc	Heated 3 hrs. at 98°C.	0.1626 0.2207	0.1626 0.2206	100.0 99.9	25 cc. C_2H_5OH added
Regular pressure	60–70 cc.	30 cc.	Heated 3 hrs. at 98°C.	0.3003	0.2995	99.7	No change in method

tetrachloride in the same manner as Sample No. 1 (chloroform). The filtered sample was distilled into a cooled brown bottle at a temperature not exceeding 76°C. The following tests were then made on the purified sample:

TESTS ON COLLABORATIVE SAMPLE NO. 2—CARBON TETRACHLORIDE

Specific gravity... .... See Table 1

Non-volatile residue at 100°C. (U. S. P. limits 0.001g./50 cc.) . . . 0.0000

Chlorides, free chlorine, U. S. P. tests	None
Aldehydes, readily carbonizable substances, U. S. P. tests	None
Carbon disulfide, U. S. P. test	Trace

TABLE 3.

Specific gravity data on pure chloroform and carbon tetrachloride compared with the collaborative samples.

AUTHORITY	MATERIAL	SPECIFIC GRAVITY 25°/25°C. IN AIR	COMMENT
Thorpe. *J. Chem. Soc.*, 1880, **37**: 196.	Pure chloroform	1.4839*	
Timmermans. *Compt. Rend.*, 1922, **174**: 365.	Pure chloroform	1.4837*	
Thorpe	Pure chloroform	1.4836*	
Newcomb. *Analyst*, 1926, **51**: 19.	Pure chloroform	1.4833*	By Westphal balance
Moraw	Pure chloroform—A. O. A. C. Sample No. 1, alcohol free	1.4844 1.4839 1.4833 1.4831 1.4829	By pycnometer By pycnometer By Westphal balance By Westphal balance By Westphal balance
Moraw	Pure chloroform containing 0.5 per cent alcohol—A. O. A. C. Sample No. 1	1.4774 1.4769 1.4763 1.4761 1.4759	By pycnometer By pycnometer By Westphal balance By Westphal balance By Westphal balance
U. S. Pharmacopeia	Chloroform containing 0.5 per cent alcohol	1.474 to 1.478	
Moraw	Pure carbon tetrachloride—A. O. A. C. Sample No. 2	1.590 1.589 1.5889	By pycnometer By Westphal balance By Westphal balance
U. S. Pharmacopeia	Pure carbon tetrachloride	1.588 to 1.590	
Annual Reports of the Chemical Laboratory of the American Medical Association, 1923, p. 42.	Carbon tetrachloride	1.5888 1.5889	Pure redistilled C. P. medicinal

* Calculated by associate referee to 25°/25°C. in air.

The authentic data on the specific gravity of pure chloroform or carbon tetrachloride are limited, none being available on chloroform at the temperature of 25°C., which has been adopted by the Pharmacopeia as the working temperature. It is for this reason and the fact that the gravity is a valuable guide in judging purity that the data in Table 2 are given. The figures of Thorpe and Timmermans for pure chloroform at 0°/4°C.

and 11.8°/4°C. in vacuo were converted to specific gravity at 25°/4°C. in vacuo by using Thorpe's figures for rate of change of density per degree change of temperature. These values were then corrected to the condition of sp. gr. 25°/4°C. in air by applying the usual correction for the buoyancy of air. The results thus obtained were further calculated by the usual procedure to the condition of 25°/25°C. in air.

$dD/dt =$ −0.00188 at 0°C., −0.00190 at 20°C.
−0.00189 at 10°C., −0.00192 at 30°C.

The specific gravity in air at 25°/25°C. of the A. O. A. C. Sample No. 1 chloroform is the only result that is a direct determination. The literature that contained Thorpe's and Timmermans' figures for specific gravity of pure chloroform[1] does not state that they are for vacuo. Newcomb states that his figure at 27°C. was for vacuo and represents grams per milliliter. The close agreement between the gravities given by Thorpe and Timmermans, when calculated from the condition of vacuo to 25°/25°C. in air, tends to confirm the assumption that they were taken in vacuo. The specific gravity of the A. O. A. C. chloroform and carbon tetrachloride samples is in sufficiently close agreement with the data on pure products to indicate that they were of the highest purity attainable.

COMMENTS BY COLLABORATORS.

P. W. Morgan: The results obtained by this method are all that may be desired. Complete diffusion of the contents of the weighing bottle is best accomplished, in my opinion, by inserting into the weighing bottle a narrow glass tube attached to a wash bottle, and flushing the contents of the bottle into the volumetric flask.

H. Wales: The chloroform results show excellent agreement. Those on carbon tetrachloride, while sufficiently accurate for drug analysis, indicate that some further modification of the method may be necessary. Diffusion of the contents of the weighing bottle is best accomplished by allowing it to stand overnight in the 200 cc. of water. The results for carbon tetrachloride were obtained by using Lintner pressure bottles, but almost any method is better than one using pressure bottles. Because these bottles are used at a higher temperature than that at which they are tested, the unequal expansion of the glass and metal portions may cause a leak that will escape detection.

L. E. Warren: I do not consider the use of a pressure bottle necessary.

Peter Valaer: It would seem that a larger portion for analysis would be necessary so as not to multiply the error so many times. The difference of 0.1 cc. in the titration made appreciable differences in the final percentages.

DISCUSSION OF RESULTS.

Since no collaborative work was done on the separation of chloroform from mixtures, the discussion will be confined to the estimation of chloroform and carbon tetrachloride. A method for the separation of chloroform from mixtures will be proposed for further study.

There are two points in the proposed method for the determination, the necessity for which has been questioned by collaborators, and it

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[1] U. S. Bur. Standards Circ. No. 19.

TABLE 4.

Collaborative results on chloroform and carbon tetrachloride.

COLLABORATOR	SAMPLE NO. 1—CHLOROFORM 99.5 PER CENT BY WEIGHT	SAMPLE NO. 2—CARBON TETRACHLORIDE, PURE
	per cent	*per cent*
Blaisdell and Bradshaw		99.56 99.41 99.24
Peter Valaer, Jr.	96.9 98.55 96.01 99.95 99.95 98.6 99.25 99.87	
P. W. Morgan	100.4 99.06	99.61
H. Wales	99.7 99.36 99.58	100.0 98.4 99.1
Elemer Schulek*	99.4 99.85 99.5	99.5 99.7
L. E. Warren	98.15 97.58 (99.86)† (96.70)† (99.38) (98.24) 100.21‡ (100.70)†‡	98.83 (98.45)† 97.05 (97.89)† 100.24 (100.06)†
H. O. Moraw	99.6 99.9 99.7	99.7 99.9 100.0

would be desirable to eliminate them if complete recovery could be obtained in any other way. These points are:

1. The use of pressure bottles.
2. Heating to complete the reaction.

In this connection a large number of determinations were made by the associate referee, as shown in Table 2, to ascertain whether complete recovery could be obtained without heating, but by using pressure bottles. The recovery varied from 96.98 to 98.8 per cent for chloroform and 6.5 to 61.1 per cent for carbon tetrachloride, without heating. By heating, according to the proposed method, it will be seen from the results in Table 2 that complete recovery was obtained. One of the collaborators, Warren, reported as his last result on the chloroform sample 100.2 per cent and a duplicate of 100.7 per cent without heating. This cannot be accounted for as the associate referee tried at least six determinations, allowing one to stand over the week-end, and the highest recovery obtained without heating was 98.8 per cent on chloroform and 61.1 per cent on carbon tetrachloride.

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* Assistant Professor of the Royal Hungarian University. At the Chicago Station under the auspices of the Rockefeller Foundation.

† Duplicate determinations on those not in brackets.

‡ These results obtained by saponification in the cold.

Based upon these experiments, it is the opinion of the associate referee that heating is necessary to obtain complete recovery, both on chloroform and carbon tetrachloride, and that it must be applied under pressure.

The directions for the estimation, as recommended for adoption last year, are satisfactory as they stand, with one exception. They should provide for the addition of alcohol in the case of carbon tetrachloride, if necessary, to effect solution of the latter in the cold. It was noticed that in the cold this compound does not go into solution in the reagent. Of course it dissolves when warmed, as provided in the directions, and the results are not influenced by this condition, but it would seem desirable to have the carbon tetrachloride in solution.

The collaborative results on the estimation given in Table 4, except for two or three of Valaer's and Warren's, are in good agreement and show complete recovery. Valaer obtained a sufficient number of check results to have eliminated the low ones. Some of the disagreement in Warren's results is due to failure to check on aliquot determinations and cannot be attributed to the saponification.

The method for the estimation appears to be satisfactory, without further study. Directions are submitted herewith for the separation of chloroform from mixtures.

RECOMMENDATIONS[1].

It is recommended—

(1) That the method for the estimation of chloroform and carbon tetrachloride be adopted as a tentative method. (This method has been published.[2])

(2) That the following directions for the separation and determination of chloroform in mixtures be submitted for collaborative study next year, and the quantitative separation with known samples be made the objective.

CHLOROFORM AND CARBON TETRACHLORIDE—TENTATIVE.

REAGENTS.

(a) *Alcoholic potassium hydroxide.*—Dissolve 30 grams of potassium hydroxide in 30 cc. of water. Cool, and dilute to 100 cc. with methyl alcohol

(b) *0.1 N silver nitrate solution.*

(c) *0.1 N ammonium or potassium thiocyanate solution.*—Adjust by titrating against the 0.1 *N* silver nitrate solution.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10** 68

[2] *This Journal*, 1926, **10**. 45

(d) *Nitric acid.*—Free from the lower oxides by diluting strong nitric acid with water (4 + 1) and boiling until colorless.

(e) *Ferric indicator.*—A saturated solution of ferric ammonium alum.

PREPARATION OF SAMPLE.

Separation of chloroform in mixtures: To the accurately weighed or measured sample containing 0.2–0.5 gram of chloroform, add, if not present already, sufficient alcohol and water to produce a mixture of 50–75 cc. containing about equal parts of the alcohol and water. Make acid with sulfuric acid. Distil at first with a low flame through a long condenser connected to an adapter that dips into the 30 cc. of reagent in the pressure bottle, which is cooled in ice water. Complete the distillation in 30 to 50 minutes, raising the temperature toward the last. Wash off the adapter with a spray of alcohol. Stopper the pressure bottle and allow it to warm to room temperature. Release the pressure quickly.

Proceed to "Determination" starting with "Stopper the bottle so as to insure a tight fit".

Carbon tetrachloride in capsules: Ascertain the gross weight of a representative number of capsules. Using a pointed knife and tweezers, open the capsules under a small quantity of alcohol. Quickly transfer the alcoholic solution to a volumetric flask, washing the capsules free from carbon tetrachloride. Weigh the dried, empty capsules and calculate the average net contents. Fill the flask to the mark with alcohol at 20°C., and stopper.

Proceed under "Determination" with an aliquot of this solution instead of weighing.

DETERMINATION.

Weigh directly 0.2–0.5 gram of the sample in a ground-glass stoppered weighing bottle of 1 to 2 cc. capacity and of such shape that it can readily be inserted into a 60–75 cc. pressure bottle. (The weighing bottle used for molecular weight determinations by the Victor Meyer method is satisfactory for this purpose.) Transfer the weighing bottle with its contents to the pressure bottle containing 30 cc. of the alcoholic potassium hydroxide, and for carbon tetrachloride add an additional 15–25 cc. of alcohol to the reagent already in the bottle to insure complete solution of the sample. Remove the stopper from the weighing bottle while submerged in the reagent, by working it out with a glass rod and wash the rod with a little alcohol. Stopper the bottle so as to insure a tight fit, mix the contents thoroughly, and allow to stand about an hour with occasional shaking. Then place the bottle in a bath of water at room temperature. Invert a wire basket over the bottle and cover with a towel to prevent injury to the operator if the bottle should burst. Heat the bath gradually to boiling and maintain at this temperature for 3 hours. Cool, transfer the contents of the pressure bottle with the aid of water to a 200 cc. volumetric flask, removing and washing out the weighing bottle, fill to the mark with water, and mix. Transfer a suitable aliquot to a 400 cc. beaker, evaporate the alcohol, acidify with nitric acid, adding about 3 cc. in excess, and determine the chlorine either volumetrically by the Volhard method, or gravimetrically by precipitating and weighing as silver chloride. Make a blank test, using in the pressure bottle the same quantities of solvents and reagents as when the sample is present and apply whatever correction may be necessary. One cc. of 0.1 *N* silver nitrate solution is equivalent to 0.003979 gram of chloroform and to 0.003846 gram of carbon tetrachloride. One gram of silver chloride is equivalent to 0.2776 gram of chloroform and to 0.2683 gram of carbon tetrachloride.

The associate referee desires to express his appreciation and thanks to A. E. Paul and C. K. Glycart for their many helpful suggestions.

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REPORT ON IPECAC ALKALOIDS.

By A. R. BLISS, JR. (College of Medicine, University of Tennessee, Memphis, Tenn.), *Associate Referee.*

The associate referee regrets that his report as a whole is rather disappointing. This fact, however, is not the fault of the collaborators who carried out the actual work and reported results, and whose valuable cooperation is acknowledged and appreciated.

The collaborative work undertaken involved: I.—the *hand extraction method* II.—the *mechanical extraction method;* and III. the *U. S. P. X method.*

The samples used in the studies were two manufacturers' preparations of ipecac, both fluidextracts.

The samples were sent to four investigators, two receiving one product and two the other. Results, however, were subsequently received from but three of the collaborators.

In addition to the samples, a letter was sent to each collaborator setting forth the methods to be used and asking that results be reported in grams per 100 cc. and that comments and suggestions accompany each report.

The methods of analysis studied were the following:

FLUIDEXTRACT OF IPECAC.

PREPARATION OF SAMPLE.

Pipet 20 cc. of the sample into a small beaker. Add 5 cc. of normal sulfuric acid and evaporate on a steam bath with the aid of an air blast to a volume of about 10 cc. or less. Transfer the entire alkaloidal solution to a 100 cc. volumetric flask and add about 30 cc. of water while rotating the flask; cool, and dilute to volume. Allow to stand 5 minutes and filter.

Method No. 1—Hand Extraction.

Pipet 20 cc. of the filtrate (representing 4 cc. of the original sample) into a separatory funnel. Add 2 cc. of 8 per cent ammonium hydroxide and extract the alkaloids with equal volumes of ether at least eight times, or until complete. Test the final extracted residue with Mayer's reagent.

Transfer the ether extract to a second separatory funnel, wash with 10 cc. of water, and withdraw the ether to a beaker or 200 cc. Erlenmeyer flask. Evaporate the combined extracts on the steam bath, using an air blast. Warm the alkaloidal residue with 2-3 cc. of neutral alcohol on the steam bath to insure complete solution. Add

10 cc. of 0.1 *N* sulfuric acid, or equivalent; dilute with about 20 cc. of water; and titrate the excess acid with 0.02 *N* alkali, using methyl red indicator. One cc. of 0.1 *N* acid = 24 mg. of ether-soluble alkaloids of ipecac.

Method No. 2—Mechanical Extraction.

Pipet 20 cc. of the filtrate (representing 4 cc. of the original sample) into a mechanical extractor that has been fitted to a 200 cc. Erlenmeyer flask. (The form described by Palkin, Murray, and Watkins[1] is very satisfactory.) Add 2 cc. of dilute ammonia solution (8 per cent ammonium hydroxide) and about 25 cc. of ether. Shake gently to prevent the settling of any solid matter on the bottom of the extractor, then add ether until about 50 cc. overflows into the flask. Heat the flask on a steam bath (not electric hot plate) and extract for 2 hours, or until complete. Separate the ether from the aqueous layer and add it to the main concentrate in the flask. Evaporate the combined ether extract on a steam bath, add 2–3 cc. of absolute alcohol, and repeat the evaporation to remove all traces of ammonia. Titrate the alkaloids as in Method No. 1.

Reports were received from the following collaborators: H. O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.; C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.; Howard R. Watkins, Bureau of Chemistry, Washington. D. C. The associate referee was unable to carry on actual work himself because of the removal of his laboratories to a new building.

The results reported are given in Table 1.

TABLE 1.

Collaborative results on fluidextract of ipecac.

(Grams per 100 cc.)

ANALYST	METHOD NO. I		METHOD NO. II		U. S. P. X METHOD	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
H. O. Moraw	1.25 1.17	2.41*	1.12 1.19	. .	*	
C. K. Glycart	1.08*	2.29 2.28	....	2.34 2.38	..	1.92
H. R. Watkins	....	*	1.61 1.49		*	

* See "Discussion of Results".

COMMENTS BY COLLABORATORS.

H. O. Moraw: I also assayed the sample sent to Glycart, using Method No. 1. Since we are in the same laboratory it was agreed not to announce results until both had finished. On comparing them then, it was found that my sample contained about half the amount of alkaloids that his contained, so we exchanged samples.

I prefer Method No. 1 to the mechanical extraction or U. S. P. X method, as it can be completed more quickly and is entirely satisfactory.

C. K. Glycart—Method No. 1, hand extraction: I prefer this method because it is rapid and extraction is complete. No emulsion was encountered.

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[1] *Ind Eng. Chem.*, 1925, **17**: 612.

Method No. 2, mechanical extraction: Complete extraction was not obtained in 2 hours. It is my opinion that the slightly higher results are due to the fact that the extraction is made at a higher temperature.

U. S. P. X assay: The assay is long. I failed to get complete removal of the alkaloid even after repeated extraction.

The results of my analyses were compared with those of Moraw on completion of the work. It was found that my sample contained about twice the amount of alkaloids. An exchange of samples was made, and I checked his results by Method No. 1, hand extraction.

H. R. Watkins: The results are disappointing except for the development of a new procedure, which in special cases may replace the acid purification of the method on trial in this cooperative work.

This fluidextract contained considerable sediment, which was filtered off before attempting any assays. The acid purification process resulted in a cloudy or turbid solution, which could not be cleared by filtration. The emulsions formed by the automatic extraction process and by the separatory funnel were so obstinate that no satisfactory results were possible. The following, obtained by automatic extraction, are reported in grams per 100 cc. of fluidextract: Assay 1.61, 1.49.

The method of U. S. Pharmacopeia X is impossible of application, owing to emulsions. A radically different purification process, which seemed promising for such an intractable preparation, was attempted. This procedure gave a solution that was practically free from all emulsion when well shaken in the separatory funnel. The final residue obtained had some yellow color, and the end point of the titration was quite satisfactory. Further work on this method of purification, on a larger number of samples, is necessary to test its general applicability.

Details are as follows:—Treated 2 cc. of the sample with 10 cc. of alcohol in a 50 cc. volumetric flask and shook thoroughly. Added 1 cc. of strong ammonium hydroxide and 15 cc. more of alcohol and mixed thoroughly. Added ether to volume and shook the contents thoroughly. Filtered and evaporated 40 cc. of the filtrate to dryness, or nearly so, in a beaker. Took up in about 10 cc. of 0.1 *N* sulfuric acid, being careful to have all alkaloid in solution. (Warmed on steam bath when necessary.) Transferred the contents of the beaker to a separatory funnel, washing the beaker with a little water and also with ether. Made alkaline and shook out with four portions (25 cc.) of ether. Washed the ether extract with water and finally evaporated to dryness on the steam bath. Took up the residue in a little alcohol, being careful to get all alkaloid into solution. Added excess standard acid (0.02 or 0.1 *N* sulfuric acid) and heated the solution on the steam bath to expel the alcohol. Cooled and titrated the excess acid with 0.2 *N* sodium hydroxide, using methyl red as indicator.

Prepared 5 cc. portions of the sample similarly, using 55 cc. of alcohol (in portions), 1 cc. of strong ammonium hydroxide, and ether to 100 cc. Carried out details as described.

TABLE 2.

Results obtained by two collaborators, using Palkin-Watkins purification procedure.

(Grams per 100 cc. of fluidextract.)

ANALYST	USING 2 CC. FLUIDEXTRACT	USING 5 CC. FLUIDEXTRACT	
S. Palkin	1.74	1.62	
H. R. Watkins	1.75	1.62	1.64

Though similar to the preparation submitted last year, this year's collaborative sample seems to be much worse as regards emulsifying properties.

RECOMMENDATIONS[1].

It is recommended—

(1) That because of the incomplete collaborative reports, the studies of Methods I, II, and III be continued.

(2) That the Palkin-Watkins purification procedure given in this report by Watkins be subjected to collaborative study.

(3) That studies of the U. S. P. X gravimetric and the modified U. S. P. IX method, given in the 1925 report on ipecac alkaloids[2], be discontinued, since both methods present but slight modifications of the present U. S. P. assay, and consequently do not involve different underlying principles.

REPORT ON RADIOACTIVITY IN DRUGS AND WATER.

By J. W. SALE (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

In 1924 a procedure for the qualitative determination of radioactivity of samples in solid form and for the quantitative determination of clear solutions of drugs and water was described by the writer and adopted as a tentative method by the association. The description included a sketch of a suitable apparatus, a method of standardizing electroscopes, and a selected bibliography[3]. It did not include a method for the qualitative examination of liquid samples nor a method for the preparation of samples for analysis. Many samples of drugs and water are received in solid or semi-solid form or in the form of liquids containing suspended matter. Such samples must be carefully prepared for analysis unless they are sufficiently radioactive to be analyzed by the gamma ray scope. The objects to be attained in the preparation of the samples for analysis are clear solutions, which will remain clear when acidified with nitric acid and boiled for 20 minutes. The boiled samples, after being sealed, should remain clear for 30 days. There must not, of course, be any loss of radium during the preparation of the samples for analysis.

The procedure described below for the qualitative examination of liquid samples and for the analysis of solid, semi-solid, and liquid samples is based on the results of tests made in the Water and Beverage Laboratory and on the practical experience of C. H. Badger and the writer over a period of about 9 years in the examination of commercial samples of many types.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**: 68.
[2] *This Journal*, 1926, **9**: 302.
[3] *Ibid.*, 1925, **8**: 531.

QUALITATIVE DETERMINATION.

(For liquids.)

Determine the natural leak of the alpha ray electroscope by taking readings over the same part of the scale until the rate of fall of the leaf becomes constant. Open the bottle containing the sample and, without delay, hold the open mouth a few cubic centimeters from the charged plate of the alpha ray scope. If no effect is observed on the rate of fall of the leaf, fill a shallow dish to a depth of about 1 cm. with a portion of the well-mixed sample. An increase in the rate of fall of the leaf in either case is presumptive evidence of radioactivity. Negative results do not indicate an entire absence of radioactivity, since radium may be present in the sample in minute quantities without affecting the rate of fall of the leaf, under the above circumstances.

QUANTITATIVE DETERMINATION.

PREPARATION OF SAMPLE.

A. *Samples completely soluble in acids:*

(a) If the sample is in solid or semi-solid form, place 50 cc. of dilute nitric acid (1 + 9) on it and boil for several minutes. If a residue remains, add 50 cc. of dilute hydrochloric acid (1 + 9) and again boil. (This treatment should not be applied to samples containing grease, such as face creams, the physical appearance of which will indicate that they are insoluble in aqueous solutions.)

(b) If the sample is in liquid form, that is either a clear liquid, turbid liquid, or liquid containing suspended matter, add 50 cc. of dilute nitric acid (1 + 9) to from 1 to 10 cc. of it, boil for several minutes, and examine carefully for opalescence. If a portion of the sample remains undissolved, add 50 cc. of dilute hydrochloric acid (1 + 9) and again boil. The addition of hydrochloric acid to nitric acid mixtures in this case and in the case of samples in solid and semi-solid form should be avoided if possible.

(c) If clear and limpid solutions are obtained by the above procedures, take such a quantity of the well-mixed sample as will produce an accurately measurable increase in the rate of fall of the leaf, dissolve as directed under (b), preferably in a florence flask of 300 cc. capacity, and proceed as under C "Final preparation of clear solutions".

B. Samples insoluble or incompletely soluble in acids:

(*a*) *Preliminary treatment:*

(*1*) *Solids:* If the sample is not in powder form, grind it to a fine powder, ignite a weighed portion of it in a porcelain dish in a muffle at dull red heat, avoiding fusion, and proceed as described below under (b) "Treatment of ash."

(*2*) *Semi-solids:* Ignite quite rapidly in a muffle a weighed portion of the sample contained in a porcelain dish, avoiding fusion. Heating too slowly or heating in the open may cause the sample to "creep" over the edge of the dish. Proceed as under (b) "Treatment of ash".

(*3*) *Liquids immiscible with water:* Evaporate a weighed or measured portion of the sample to dryness, or as nearly so as possible, on a steam bath, and dry carefully on a hot plate. Ignite the residue in a muffle, avoiding fusion. Proceed as under (b) "Treatment of ash."

(*4*) *Liquids containing material which is insoluble in dilute nitric acid:* Digest the sample or a suitable portion of it, with dilute nitric acid (1 + 9). Filter into a 300 cc. florence flask, and wash the residue thoroughly with hot water. Proceed as under (b) "Treatment of ash", beginning "Ignite the washed residue in a platinum dish * * *".

(*b*) *Treatment of ash:*

(1) Digest the ash obtained under (a) with dilute nitric acid on a steam bath. Note the quantity of acid used in this and subsequent operations so that the final clear solution can be adjusted to contain about 10 per cent of acid by volume. Filter into a florence flask and wash thoroughly with hot water. (Ordinarily a flask of 300 cc

capacity is the most suitable, even if it is necessary to concentrate the filtrates by boiling.) Ignite the washed residue in a platinum dish and cover the residue with a few cc. of water and 5 to 10 cc. of hydrofluoric acid. Evaporate to dryness on a steam bath. Add water and a few cc. of nitric acid (1 + 9), digest on a steam bath, filter into a florence flask, and wash with water. Ash the filter paper in a platinum dish and add 5 to 10 cc. of water and 1 cc. of strong nitric acid. Examine carefully for any insoluble material, and if none is found, add the solution directly to the florence flask, rinsing out the platinum dish several times with water and adding the washings to the flask. Then follow the procedure under C "Final preparation of clear solutions". If an insoluble residue remains, proceed as follows:

(2) Ignite the insoluble residue in a platinum dish and fuse it with five to ten times its weight of a fusion mixture consisting of equal weights of potassium carbonate and anhydrous sodium carbonate. After cooling, neutralize the fused mass with dilute nitric acid, using a drop of phenolphthalein solution to note when the solution is acid. Heat on a steam bath, add a few cc. excess of nitric acid, and boil carefully. Filter the solution into the florence flask and wash thoroughly. Ignite the insoluble residue in a platinum dish and proceed as under (b) "Treatment of ash", beginning with "cover the residue with a few cc. of water and 5 to 10 cc. of hydrofluoric acid".

C. Final preparation of clear solutions:

Boil the clear solution obtained under A or B for 20 minutes, and dilute it with water and nitric acid to a volume of about 250 cc.; if the sample is contained in a flask of 300 cc. capacity, cool somewhat and seal without delay, noting exact time of sealing. All reagents should, of course, be free from radium or radon. Seal the flask in the following manner: Bend a piece of glass tubing at right angles, draw out one end, and seal off. The sealed-off arm should be about 15 cm. long and the other arm about 7 cm. long. Place the short arm in a one-hole rubber stopper, so that the end is flush with bottom of stopper. Place stopper and tubing in neck of flask so that a tight fit is obtained and tie down to the flask with a cord. Examine the solution the next day. If an opalescence or precipitate is noted, filter the solution, wash the insoluble residue with hot water, and treat it as described under B (2) beginning "Ignite the insoluble residue in a platinum dish and fuse it * * *".

RECOMMENDATIONS[1].

It is recommended—

(1) That the procedure described in this report for the qualitative examination of liquid samples and for the preparation for analysis of solid, semi-solid, and liquid samples be adopted as tentative.

(2) That the associate referee for next year prepare a description of the preparation of a standard stock solution of radium.

REPORT ON LAXATIVES AND BITTER TONICS.

By H. C. FULLER (Washington, D. C.), *Associate Referee.*

Attention was devoted, this year, especially to cascara sagrada. A series of tests was applied to specimens of the crude drug, fluidextract, and aromatic fluidextract. The procedure adopted was as follows:

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, 10: 68.

Treat the samples according to the method previously published, *This Journal*, 1925, 8: 537, to the point where the anthraquinones have been removed from the chloroform by means of sodium hydroxide (10 per cent).

Run the alkaline liquid into a 100 cc. flask and make up to volume with water.

Remove 5 cc. by means of a pipet and transfer to a 50 cc. Nessler tube, making up to volume with water. Make up further Nessler tubes, using 2.5, 7.5, and 10 cc. of alkaline liquid.

Compare the color imparted by a control specimen of known gravimetric assay, and from the comparisons calculate the percentage of anthraquinones in the specimen.

Use 1 gram of crude drug and 5 cc. of fluidextract for samples.

The results obtained by the gravimetric method and some of the assays by color comparison are given below. The checks were not equivalent in every one of the specimens tested.

	GRAVIMETRIC *per cent*	COLORIMETRIC *per cent*
Crude Drug No. 1	2.83	2.37
Crude Drug No. 2	3.80	Standard
Fluidextract from Drug No. 1	1.53	
Fluidextract from Drug No. 2	1.77	
Fluidextract, Manufacturer No. 1	1.13	0.95
Fluidextract, Manufacturer No. 2	0.75	0.63
Fluidextract, Manufacturer No. 3	1.05	1.18
Fluidextract, Aromatic, Manufacturer No. 1	0.32	
Fluidextract, Aromatic, Manufacturer No. 2	0.75	
Fluidextract, Aromatic, Manufacturer No. 3	0.39	

Following these experiments, a special test was made to compare the color developed by the anthraquinones from 1 gram of Crude Drug No. 2, with that developed by the anthraquinones from 5 cc. of fluidextract cascara, Manufacturer No. 2. These specimens were selected because they gave residues of nearly the same weight by the gravimetric test, namely, 0.0380 gram for the former and 0.0374 gram for the latter. It will be noted that 1 gram of the crude drug and 5 cc. of the fluidextract were used, there being approximately 5 times as much anthraquinone in the drug as in the fluidextract.

The color comparison was made as follows: The samples were treated according to the same method down to the point where the chloroform solution had been shaken out with 2 per cent sodium bicarbonate. The bicarbonate was separated and discarded. The yellow chloroform solutions were then made equal in volume with chloroform. Ten cc. portions of the solution were transferred to Nessler tubes and made up to volume with chloroform. The yellow shades matched very closely.

The balance of the yellow chloroform solution was shaken out with 5 per cent potassium hydroxide, and the alkaline liquid was collected in a 100 cc. flask and made up to volume with water.

Five cc. portions were transferred to Nessler tubes and made up to 50 cc. with water. The tints differed when viewed directly, but the intensity was very close. The color from the fluidextract was a bit paler than that given by the drug.

The tints imparted by different preparations are not uniform. They may be of the same intensity, but they differ in actual shade. It is evident that the color comparison furnishes a relatively rapid method for arriving at an approximate idea of the strength of the drugs and fluidextracts insofar as the anthraquinones are concerned, but as yet it cannot be considered accurate. In actual practice with unknowns it has given surprisingly close results, as shown by a subsequent account of the history of the specimens tested.

The question of the anthraquinone test, either gravimetric or colorimetric, as a measure of the physiological activity of cascara is still undecided. It was hoped that some significant data would be secured with the large number of observations made, but the work is incomplete.

It was found that there are variations in the individual susceptibility of human subjects, and that in order to get satisfactory comparisons, the same subject must be used to test a series of products with different anthraquinone contents. Any number of subjects can be used, but each is a separate research.

Observations have shown that, given a crude drug with an assay of 3.8 per cent, a fluidextract assaying 1.5 per cent, and an aromatic fluidextract testing 0.4 per cent, the general run of subjects requires considerably less of the fluidextract to produce good cathartic action than of the aromatic fluidextract, and still less of the crude drug to produce equivalent effects.

While the curve of these data follows in general the anthraquinone curve in an inverse manner, the comparisons are not quantitative, and it is evident that there are many factors that need intensive study.

It is considered that the gravimetric assay of cascara discussed in this report is better than any other process yet proposed for arriving at an estimate of the activity of the crude drug and of an unaltered fluidextract. That there may be constituents other than anthraquinone derivatives that are factors in producing the laxative effect, is advocated by other experimenters, and the associate referee is open minded on the subject. But intensive study leads to the belief that the anthraquinone value gives a good indication of the strength and purity of the drug and fluidextract.

The associate referee considers that the work on this subject should be continued[1].

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1927, **10**: 68.

REPORT ON MERCURIALS.

By PERRY W. MORGAN[1] (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee.*

In accordance with last year's recommendation, further study was made of the methods for the examination of mercurials.

The U. S. Pharmacopeia includes two methods for the estimation of calomel in the absence of interfering substances, namely, the iodine titration and the electrolytic methods. The iodine method is not applicable in the presence of milk sugar and starch, which are common excipients in commercial calomel tablets. No method employing iodine is applicable in the presence of these excipients.

As regards the electrolytic method, the excipients present in calomel tablets should cause no difficulty except in washing free from the precipitated mercury. This, however, is a minor detail, but it is thought that the results of the electrolytic method should be as near the theoretical as is practically possible. However, most analytical laboratories are not equipped with an electrolysis apparatus for routine work, and it is, therefore, considered advisable to develop a method for the estimation of calomel in calomel tablets that can be used as a routine method and that will not entail the use of special apparatus or reagents.

It was thought advisable to limit the work this year to methods for the estimation of calomel; therefore, little work was done with other mercurials.

A thorough review of all the literature available resulted in the selection of the method of A. W. Bender for the determination of mercuric iodide in tablets[2] as a promising method for the estimation of mercury in calomel tablets, since the excipients are essentially the same. In principle, the method employs the solution of the calomel and the hydrolysis of the starch present by means of strong hydrochloric acid containing free chlorine. The chlorine is then removed, and the mercury is estimated by the usual sulfide method. The method as submitted for collaborative work is as follows:

METHOD.

Count and weigh a representative number of tablets to ascertain the average weight. Pulverize the sample and weigh a portion of the well-mixed powder to represent 3 to 4 grains of calomel and transfer it to a 250 cc. Erlenmeyer flask. Add 20 cc. of hydrochloric acid (1 + 1) and about 0.5 gram of potassium chlorate. Cover the flask with a small watch glass and allow the mixture to digest at room temperature for about 30 minutes. Place the flask on a steam bath and digest for 30 minutes more. Dilute the solution to about 75 cc., aspirate to remove the free chlorine, and filter into a 250 cc. Erlenmeyer flask, washing well with water until the volume is 150–175 cc. Add about 7 cc. of concentrated ammonium hydroxide water until the acid is almost neutralized, pass hydrogen sulfide through the solution for 30 minutes, and warm to 80°C. for 5 minutes, or until the precipitate settles quickly after shaking. Pass hydrogen sulfide into the warm solution again for 5 minutes. Filter the solution immediately into a weighed Gooch crucible, and wash the filter well with water, then three times with alcohol (95 per cent) in 10 cc. portions, and finally with carbon tetrachloride or carbon bisulfide to remove any sulfur that may be present. Dry the precipitate to constant weight at 110°C. and weigh it as mercuric sulfide. The weight of mercuric sulfide multiplied by 1.0146 gives its equivalent in calomel.

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[1] Presented by E. K. Nelson.

[2] *J. Ind. Eng. Chem.*, 1914, 6: 753.

RESULTS.

A sample of calomel, complying with the U. S. P. and other available tests, was used as it was considered of sufficient purity for this work.

A sample containing 10 per cent calomel, 50 per cent lactose, and 40 per cent starch was prepared and well mixed. The mercury was estimated by the method given and the following results were obtained:

ANALYST	WEIGHT OF SAMPLE	WEIGHT OF HgCl FOUND	PERCENTAGE
P. W. Morgan	*gram* 0.5000 2.0600	*gram* 0.0502 0.2050	 10.04 9.95
Elemer Schulek* Royal Hungarian University Budapest, Hungary	1.0245 2.0521	0.1045 0.2083	10.20 10.15
H. O. Moraw U. S. Food and Drug Inspection Station Chicago, Ill.	1.5000 1.5000	0.1520 0.1498	10.13 9.99

* A Rockefeller Institute visitor in the United States.

In order to obtain further indication of the accuracy of the method, the associate referee weighed two samples of calomel and added the proportional amounts of lactose and starch afterwards. The samples were then analyzed as before. The results are as follows:

WEIGHT OF SAMPLE, PURE CALOMEL	CALOMEL FOUND	
gram	*gram*	*per cent*
0.2000	0.1994	99.70
0.2000	0.2017	100.85

COMMENTS BY COLLABORATORS.

Elemer Schulek: I found that this method gives consistent results and since the manipulation is simple, it is an excellent method for routine analysis.

H. O. Moraw: It is believed that the directions for washing with carbon tetrachloride to remove sulfur should be given with more detail, as in the U. S. Pharmacopeia under "mercuric chloride," for example.

I have had trouble with mercuric sulfide precipitates running through the Gooch crucibles and believe that the asbestos mat may have been too thin. Since sulfide precipitates are recognized in the literature as being hard to filter quantitatively, might it not be well to specify a thick mat to insure better results and eliminate trouble?

DISCUSSION.

It is the opinion of the associate referee that the method is correct in principle and that the results are within the limits of accuracy desirable for quantitative and regulatory work.

The excipients used by reputable manufacturers are of such nature as to cause no difficulty with the estimation of the mercury by the method given in this report. The method has been used by the associate referee for the estimation of mercury in commercial tablets of calomel, mercuric iodide, and mercurous iodide, and has produced consistent results. It is thought that with a few changes the method will give results on mercuric and mercurous iodides equal to those obtained upon calomel. With slight modifications and additions, the method may also be adapted to the determination of mercury in the presence of copper, bismuth, and arsenic.

RECOMMENDATIONS[1].

It is recommended—

(1) That the method as outlined be adopted as tentative for the estimation of calomel in tablets.

(2) That the method be further studied for the purpose of determining what modifications or changes are necessary for the determination of other mercurials, or in the presence of copper, bismuth, and arsenic.

REPORT ON PYRAMIDON.

By WILLIAM RABAK[2] (Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee.*

The method submitted and tested by the associate referee last year for the quantitative determination of pyramidon was found to yield favorable results. This method, which is a modification of the present tentative extraction method (I)[3], has been published[4]. Accordingly, at the suggestion of the Referee on Drugs, the method was studied this year in a collaborative way. The results of four collaborators are shown in the table.

From the results given it will be noted that in the case of Sample No. 1 the average recovery was 100.9 per cent, Sample No. 2—100.5 per cent, and Sample No. 3—100.0 per cent.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**. 68.
[2] Presented by L. N. Markwood.
[3] *This Journal*, 1925, **8**: 546.
[4] *Ibid.*, 1926, **10**: 309.

COLLABORATIVE RESULTS.

COLLABORATOR	SAMPLE NO. 1 60% PYRAMIDON 40% MILK SUGAR	SAMPLE NO. 2 70% PYRAMIDON 30% MILK SUGAR	SAMPLE NO. 3 50% PYRAMIDON 50% MILK SUGAR
	per cent	*per cent*	*per cent*
Ko Suto	63.45	72.40	52.40
	64.35	73.40	51.40
W. F. Kunke	59.60	70.00	49.80
	60.30	70.00	49.30
	60.80	70.10	49.20
	60.60	70.10	49.60
	60.50	70.40	49.50
L. H. McRoberts	60.40	70.35	50.50
	58.50		50.10
P. J. Valaer	58.60	69.00	49.50
	59.10	68.30	48.80
		69.90	49.90
L. Burritt			
——Ettienne			
Average	60.56	70.36	50.00

RECOMMENDATION[1].

In view of the excellent results brought out by collaborative work, it is recommended that this method be adopted and that no further work be inaugurated on the assay of pyramidon.

REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS.

By CHRIS K. GLYCART[2] (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee.*

Several publications on the subject of the identification of alkaloids[3] and other drugs[4] by microchemical methods have appeared since 1869. In 1909 and 1911, Howard and Stephenson[5] contributed to this association, reporting the progress of their investigations on sixty-seven alkaloids. The complete details of this extensive study were published in book form by C. H. Stephenson[6].

With the exception of diacetylmorphine[7], no microchemical tests for alkaloids have been adopted by the association. At the suggestion of the Referee on Drugs, the preliminary study here reported was made.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**: 69.

[2] Presented by J. F. Clevenger.

[3] T. G. Wormley. Micro-chemistry of Poisons, 1869, 2nd ed. 1885; E. B. Putt. Microchemical Tests for the Identification of Some of the Alkaloids. *J Ind. Eng. Chem.*, 1912, **4**: 508.

[4] L. E. Warren. *J. Am. Pharm. Assoc.*, 1923, **12**. 512.

[5] B. J. Howard and C. H. Stephenson. U. S. Dept Agr. Bur. Chem. Bull. 122, p. 97; Ibid., 137, p. 189.

[6] Some Microchemical Tests for Alkaloids J. B. Lippincott Co., 1921.

[7] *This Journal*, 1921, **5**: 150.

Nothing new is claimed. The literature was consulted, and the material of C. H. Stephenson and E. B. Putt was considered of especial value.

The work this year was limited to the study of five alkaloids. Directions and descriptions for the tests, control specimens of cocaine, codeine, heroine, morphine, and strychnine, and unlabeled samples of tablets were sent to the following collaborators: Percy Tarver, Department of Public Health and Welfare, Cleveland, Ohio; P. W. Simonds, Treasury Department, Chicago, Ill.; and C. H. Stephenson, Microchemical Laboratory, Bureau of Chemistry, Washington, D. C.

The unlabeled samples submitted for identification consisted of commercial hypodermic tablets of alkaloidal salts, respectively: (1) heroine, (2) cocaine, (3) morphine, (4) codeine, and (5) strychnine.

It may be stated that an attempt was made to reduce the number of reagents used by selecting those that produced the characteristic tests only. The description of the crystals was also shortened, but experience of the microscopist with the appearance of crystals obtained with the control specimens was considered important. For routine work it was found that the milk sugar in hypodermic tablets did not interfere with the formation of the crystalline precipitates.

METHOD.

REAGENTS.

Marme's		*Wagner's*	
Cadmium iodide	3 grams	Iodide	1 gram
Potassium iodide	6 grams	Potassium iodide	5 grams
Water	18 cc.	Water	100 cc.

Platinic chloride.—5 per cent solution.

Potassium permanganate.—5 per cent solution.

PREPARATION OF SAMPLE.

1. *Control.*—Dissolve a milligram of the pure alkaloidal salt in 2 drops of water to make approximately 1 to 100 solution. Proceed to microchemical identification.

2. *Alkaloids in compounds.*—Separate the alkaloid in pure form by shaking from ammoniacal solution with suitable immiscible solvents and evaporate. To a milligram of the residue add, by drops, 0.1 *N* hydrochloric acid, but avoid excess of acid. Maintain approximately the same strength of solution as in the control test.

3. *Hypodermic tablets.*—Dissolve a portion of the tablet in water, maintaining the same strength as in the control. Proceed to microchemical identification.

MICROCHEMICAL IDENTIFICATION.

Place a drop of the alkaloidal solution on a clean glass slide and add one drop of reagent by means of a clean glass rod. Without stirring or covering, examine under microscope, using low power. Note the formation of crystals and compare with description of characteristic tests for alkaloids. Magnification of 100 to 150 is suitable.

Characteristic microchemical tests for alkaloids.

Cocaine	*Platinic chloride* Delicate, feathery crystals, becoming heavier in structure.	Confirmatory test: Potassium permanganate purple plates.
Codeine	*Marme's reagent* Silvery circular masses, crystallizing into dark rosettes of irregular outline.	*Wagner's reagent* Heavy red-brown precipitate, crystallizing very slowly in yellow blades extending in branches (never red).
Heroine	*Platinic chloride* Spherical clusters of golden yellow needles slowly form around a nucleus.	
Morphine	*Marme's reagent* Silvery gelatinous precipitate, crystallizing in dense masses of fine needles.	*Wagner's reagent* Small drop of reagent produces heavy red-brown precipitate, slowly crystallizing in shining red overlapping plates extending in branches.
Strychnine	*Platinic chloride* Crystals form immediately in clusters and singly in small wedge-shape needles, which move about the field.	

The results, descriptions, and comments of the collaborators are as follows:

Percy Tarver:

Cocaine:

Platinic chloride.—Feathery crystals develop almost at once, usually much more on one side than the other, in some cases even having the appearance of a two-pronged carpet tack.

Codeine:

Marme's reagent.—Round white masses at first. Later crystals in irregular tufts, unlike heroine, which yields perfectly straight needles. Sometimes small individual triangular crystals may form.

Wagner's reagent.—After a time yellowish brown (with a slightly greenish cast) groups of crystals develop. The ends of these are usually somewhat pointed, unlike morphine, which are nearly square and red brown in color.

Heroine:

Platinic chloride.—Fine straight needles radiating from the center to a perfectly circular circumference.

Morphine:

Marme's reagent.—Gelatinous white precipitate at first, changing to fine silky needles, the longer ones showing a tendency to curve.

Wagner's reagent.—In more concentrated solutions a tree-like form, with branches continually subdividing, starts from a nucleus. The reddish brown color suggests certain species of sea weed, the large blades being on the edge of the growth. In groups more slowly formed, distinct rectangular-like crystals are found, which in overlapping one another often produce the effect of a flight of steps. The ends of such groups of crystals, therefore, are of a square type, and not acute angled as in the case of codeine. The color of the morphine compound is a red brown (burnt sienna), quite distinct from the yellow brown of codeine.

Strychnine:

Platinic chloride.—Characterized by very thin crystals arranged singly and in groups, which float on the surface of the drop. These are often wedge-shaped, or have wedge-shaped markings. Sometimes a group of rather heavy needles occurs.

Marme's reagent produces characteristic rosettes with a multigranular nucleus.

Results of microchemical identification tests for unknown tablets, submitted by Tarver.

NO.	REACTIONS		INFERENCE
1	*Platinum chloride* . .	Fine needles radiatng from center in perfect spherules	HEROINE
	Marme	White precipitate amorphous	
	Wagner	Precipitate amorphous	
2	*Platinum chloride* . .	Feathery crystals, uneven development	COCAINE
	Potassium permanganate	Floating purple rosettes	
	Wagner	Precipitate amorphous red-brown drops	
	Marme	White precipitate amorphous	
3	*Platinum chloride* . .	Amorphous droplets	MORPHINE
	Marme . .	Gelatinous precipitate, developing into long silky needles, some curving	
	Wagner .	Red brown blades, some in step form	
4	*Platinum chloride* .	Precipitate amorphous	CODEINE
	Marme . . .	Rough spheres, crystallizes in tufts, not straight needles like heroine	
	Wagner . .	Yellow-brown crystals, not reddish. End crystals usually pointed	
5	*Platinum chloride* . .	Thin wedge-like crystals, some in groups. Float on surface	STRYCHNINE
	Marme	Groups of scimitar-like blades and many rosettes	
	Wagner.	Precipitate develops short individual needles	

Paul W. Simonds:

1. *Heroine hydrochloride.*—Method very satisfactory. Crystals resemble gooseberries.

2. *Cocaine hydrochloride.*—Characteristic crystals form at once with platinic chloride and less readily with permanganate.

3. *Morphine sulfate.*—Marme's reagent forms crystals in very dilute solution. Best observed with field darkened. Tenth normal iodine solution seems to be more satisfactory than Wagner's reagent as a confirmatory test.

4. *Codeine sulfate.*—Marme's reagent satisfactory. With Wagner's reagent crystals appear to form more readily in a solution of about 1 : 500.

5. *Strychnine sulfate.*—Method very satisfactory.

C. H. Stephenson:

1. One tablet used. Not completely dissolved in three drops of water. Some corn starch present. Is heroine. With platinum chloride the crystals of needle rosettes are quickly formed. Time to make test, 2 minutes.
2. One tablet completely dissolved in three drops of water. Small amount of corn starch present. Cocaine easily determined with both chloride and platinum chloride. Time used, 5 minutes.
3. One tablet not completely dissolved in water. Morphine determined with Marme's reagent in 2 minutes.
4. One tablet not completely dissolved in water. Codeine determined with Marme's reagent in 2 minutes.
5. One tablet not completely dissolved in water. Strychnine determined with gold chloride and platinum chloride in 1 minute.

Descriptions of all crystals are given in my book. These determinations are all exceedingly easy, and I would suggest that they be given to a dozen or more chemists who have never used any microchemical tests to prove that no special training is needed to make use of such tests.

DISCUSSION.

With regard to the identification of the alkaloids in the unknown tablets, the findings of the collaborator were correct. In addition to the description, Tarver submitted diagrams of the crystals sketched in colors. In the opinion of the associate referee these have a decided advantage over photomicrographs in which much of the detail is lacking by comparison. This is particularly true in the reproduction of the appearance of overlapping crystals as in morphine and codeine by the addition of Wagner's reagent. It may be stated that a number of photomicrographs of crystals were taken for exhibit purposes, but the results were variable, and only in a few instances successful.

RECOMMENDATION[1].

It is recommended that further study be given to microchemical methods for alkaloids with the view to including a systematic description and diagrams of the more important ones.

REPORT ON SILVER PROTEINATES.

By LLEWELYN JONES[2] (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee.*

In accordance with the approved recommendations of last year, further study was given to the method for ionizable silver by yeast. For the work this year the method for ionized silver by dialysis, now official first action, was used as a basis of comparison. This method appeared in the report on Silver Proteinates given by Eaton[3], and the method by yeast was presented by Hitchcock[4].

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, 10: 69.
[2] Presented by V. K. Chesnut.
[3] *This Journal*, 1925, 8: 551.
[4] *Ibid.*, 1926, 9: 314.

Before preparing samples for collaborative examination, the associate referee, because of his limited experience with dialysis methods, carried on some preliminary work with the present official method. Considerable difficulty was experienced, partly due to the paper used, but more particularly to the rather ambiguous description of the details, as they appear at present. Four different types of paper were tried, and it was found that three were unsatisfactory, because there was more or less leakage of the silver solution. It is considered that the selection of a satisfactory grade of paper is of prime importance in carrying out this method. The instructions for the preparation of the tube should be made clearer, so that an analyst unfamiliar with this type of method may not fall into the same error as did the associate referee when he first undertook this work. The following change in the directions is suggested: In place of the sentence "Fold a square piece * * * of the proper size", substitute the following: "Over one end of a glass tube 4 inches long and approximately 1 inch in diameter fold, and secure by means of a rubber band, a square piece of parchment paper in the form of a sack, of sufficient size to hold the sample solution".

It is regretted that the preliminary work performed made it impossible for the associate referee to devote attention to the matter of alkalinity of this class of products, as was suggested by previous associate referees.

Three commercial samples, representing the different types of silver compounds, were purchased for analysis. They were labeled, respectively, "Silver Proteinate", "Silver Nucleinate", and "Collargolum".

The results of analysis are as follows:

ANALYST	SILVER PROTEINATE		SILVER NUCLEINATE		COLLARGOLUM	
	Active silver by yeast	Ionic silver by dialysis	Active silver by yeast	Ionic silver by dialysis	Active silver by yeast	Ionic silver by dialysis
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
C. K. Glycart	1.20	. . .	0.18	.	0.37	. .
L. Jones	1.32	1.27	0.22	None	0 36	None
	1.33	1.25	0.22		0.36	

COMMENTS BY COLLABORATORS.

C. K. Glycart: The method by yeast is considered a rapid and sensitive determination for differentiating between the various types of the silver proteinates. The apparatus is not complicated and the determination required approximately 2 hours.

DISCUSSION OF RESULTS.

Owing to the time consumed in the preliminary work, it was not possible to send material to many collaborators. However, C. K. Glycart kindly agreed to collaborate. His results are in satisfactory agreement with those of the associate referee. In fact, it is considered that the work done last year and this year is quite sufficient to demonstrate the value of the yeast method. Reasons for the desirability of this type of method for the examination of silver proteinates were given last year by the associate referee as well as by the referee. Under the circumstances, it is considered that the tentative adoption of the yeast method, at this time, is warranted and desirable.

RECOMMENDATIONS[1].

It is recommended—

(1) That the present official method be finally adopted, but that the description of the dialyzing tube be amended as suggested in this report.

(2) That the yeast method be adopted as a tentative method.

(3) That the question of alkalinity or acidity be given attention, and especially that the details proposed by Eaton be given collaborative study.

NITROGLYCERIN.

Report by ALFRED W. HANSON[2] (U. S. Food and Drug Inspection Station, Minneapolis, Minn.), *Associate Referee.*

Collaborative work was continued on the determination of nitroglycerin after extraction with ether and alcohol by Method No. 2 submitted by the associate referee in 1925[3].

Results reported under modification A in this report were obtained when Method 2 was used after extracting the nitroglycerin with ether.

Results reported under modification B were obtained by the alcohol aliquot method, also given in the 1925 report[4]. The directions sent to collaborators this year were as follows:

Weigh out about 15 grams of the finely powdered mixture. Transfer it to a 200 cc-volumetric flask. Add 95 per cent alcohol, shaking the sample during the addition. Dilute with alcohol to 200 cc. and allow the mixture to stand, shaking it occasionally during 1 hour. Filter through a quantitative filter paper and determine the nitroglycerin in a 25 cc. aliquot of the clear alcoholic solution. The aliquot should contain about ½ grain (0.0324 gram) of nitroglycerin. Make the determination by the Proposed Devarda Method No. 2, mentioned previously, commencing at the point where the alcoholic solution is transferred to the Kjeldahl flask.

As the alcohol aliquot method, B, was not corrected for the volume occupied by the insoluble portion of the sample, the Referee on Drugs suggested the following modification, C, which was studied by the collaborators:

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**: 69.
[2] Presented by H. C. Fuller
[3] *This Journal*, 1926, **9**: 319.
[4] *Ibid.*, 322.

C.—Count and weigh a number of tablets corresponding to 1 grain of nitroglycerin. Transfer to a glass-stoppered Erlenmeyer flask. Add 50 cc. of U. S. P. 95 per cent alcohol by means of a pipet. Reduce the tablets to a fine powder with a glass stirring rod. Stopper the flask and shake the mixture. Allow to settle. Transfer a 25 cc. aliquot of the clear solution to an 800 Kjeldahl flask. Dilute with distilled water to about 300 cc.; then proceed as under Method 2, starting "Place the flask on an asbestos centered wire gauze".

SAMPLE SUBMITTED.

The sample sent to the collaborators was prepared by mixing 20 grams of a nitroglycerin mixture containing 9 per cent nitroglycerin used for the preparation of nitroglycerin hypodermic tablets with 60 grams of milk sugar. The mixture was calculated to contain 2.25 per cent of nitroglycerin. This concentration corresponds to the quantity usually found in commercial tablets.

COLLABORATORS.

Reports were received from the following collaborators:

E. L. Anderson, U. S. Food and Drug Inspection Station, Baltimore, Md.
E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif.
C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.
A. W. Hanson.
H. O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.
H. Wales, Bureau of Chemistry, Washington, D. C.

RESULTS OF ANALYSIS.

The following results were obtained:

COLLABORATOR	A	B	C
	per cent	*per cent*	*per cent*
E. L. Anderson	1.95	2.32	2.23
	2.00	2.34	2.25
	2.07		2.27
	2.01		
E. O. Eaton	2.17	2.31	2.18
	2.16	2.32	2.19
C. K. Glycart	2.133	2.307	2.187
	2.144	2.2996	2.219
A. W. Hanson	2.13	2.29	2.21
	2.14	2.28	2.23
H. O. Moraw	2.08	2.22	2.14
	2.04	2.19	2.16
H. Wales	1.97	2.13	1.82
		2.24	2.07
		2.08	
		2.24	

COMMENTS BY COLLABORATORS.

Anderson points out that the alcoholic extraction method would give high results if other alcohol-soluble material were present. He further states that he believes Method C gives more accurate results than Method B and that both of these methods present a considerable advantage because they eliminate the tedious ether extraction from dry and wet powder.

Eaton states that Method A seems somewhat complicated and is probably no better than Method C. He states that in Method B true aliquots are not taken because the volume of the undissolved lactose cannot be calculated, and that Method C seems preferable as to simplicity. In Method C a true aliquot is taken, and this method should be satisfactory.

Glycart states that Method C is a rapid and accurate method, and that the higher results obtained by Method B are no doubt due to the space occupied by the milk sugar.

DISCUSSION OF RESULTS.

Method C was found to be a rapid and accurate method for the determination of nitroglycerin. The associate referee believes that in nitroglycerin tablets that can be reduced to a fine powder it will be satisfactory to determine the nitroglycerin by Method C. If the tablets are suspected to contain any other alcohol-soluble nitrogenous material, it would also be desirable to determine the nitroglycerin by Method A.

RECOMMENDATION[1].

It is recommended that Methods A and B be adopted as tentative methods for the determination of nitroglycerin.

REPORT ON TERPIN HYDRATE.

By C. W. HARRISON[2] (Food and Drug Inspection Station, Baltimore, Md.), *Associate Referee.*

An attempt was made to get a satisfactory extraction method that would remove terpin hydrate from solutions similar to those in which it is ordinarily dispensed. No satisfactory results were obtained. Even when the separation had been effected, the method for the determination of terpin hydrate produced no satisfactory results.

Attempts were also made along several different lines to devise a method that would give a more satisfactory quantitative determination, that is to separate the terpin hydrate by the formation of some insoluble compound so that it would be possible to weigh it. So far, however, these results have also been unsuccessful.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**: 6.

[2] Presented by A. E. Paul.

The work has been of such a nature that a complete report at this time would be useless. It is deemed desirable to continue the work for another year[1].

A search of the literature was made to find some method on which further work might be done. However, none could be found. It seems that other investigators have encountered difficulties similar to those met by the associate referee.

REPORT ON APOMORPHINE HYDROCHLORIDE.

By L. E. WARREN (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

Apomorphine is used in therapy as an expectorant and as a rapid and certain emetic. For the induction of emesis the hydrochloride of the alkaloid is chiefly employed hypodermatically. Because of the great degree of dependence that is placed on apomorphine by the physician in emergencies the need for accurate dosage is evident.

The salts of apomorphine are prone to change by exposure to light and air, green colored decomposition products resulting. The isolated base being even more subject to oxidation than the salts, the usual methods of analysis for alkaloids in tablets do not give satisfactory results with tablets of apomorphine hydrochloride.

Eaton and Murray[2] devised a method for the determination of apomorphine hydrochloride in tablets that does not involve the use of heat or undue exposure to the air by evaporation of the immiscible solvent. The method consists in extracting the alkaloid with an immiscible solvent in the presence of sodium bicarbonate, washing the solvent with water, and extracting it with a measured volume of standardized acid. The acid is drawn off, and the excess is titrated in the usual way. Eaton and Murray found the method worked well on tablets of apomorphine hydrochloride, as well as on those of some other alkaloids. They assayed a commercial specimen of apomorphine hydrochloride and found 98.1 per cent and 98.7 per cent of theory, respectively.

Since the method appeared to possess promise, the subject was taken up by the A. O. A. C., and a report was submitted by C. K. Glycart, associate referee[3]. In the collaborative work for 1925 ether was used as a solvent in place of the chloroform-ether mixture recommended by Eaton and Murray, and potassium bicarbonate was employed to liberate the alkaloid instead of sodium bicarbonate, which had also been recommended by Eaton and Murray. In the 1925 studies two preparations were assayed—one consisting of one part of apomorphine hydrochloride and two parts of milk sugar and the other of crystallized apomorphine hydrochloride of the market. The results obtained by five collaborators varied from 29.5 to 34.8 per cent of apomorphine hydrochloride for the 33⅓ per cent mixture and from 95.8 to 99.2 per cent for the market specimen of the salt. The general criticism was that owing to the tendency of the solutions to become colored the end point could not be ascertained accurately. In view of the rather wide variations in the results obtained it was recommended that the subject be studied further.

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[1] For report of Sub-committee B and action of the association, see *This Journal*, 1927, **10**: 69.
[2] *This Journal*, 1925, **8**: 572.
[3] *Ibid.*, 1926, **9**: 323.

Owing to the pressure of other duties the associate referee appointed at the 1925 meeting was unable to take charge of the project, and it was assigned to the writer quite late in the year. In correspondence with J. Rosin of the Powers-Weightman-Rosengarten Company, it was learned that he preferred to use ether as the solvent rather than chloroform, and to evaporate the ether after the addition of a measured volume of standized acid. The titration of the cooled solution was then completed in the usual way.

Two methods were studied in the collaborative work this year. In one chloroform was used as a solvent, and the assay was completed according to the procedure outlined by Eaton and Murray; in the other, ether was used as a solvent, and the solvent was evaporated according to the suggestion of Rosin. Sodium bicarbonate was used for the liberation of the alkaloid. As carried out, the methods are given herewith.

METHOD I.—ASSAY OF APOMORPHINE HYDROCHLORIDE USING ETHER AS SOLVENT.

Weigh a number of tablets equivalent to about 0.065 gram of the alkaloid or its salt and dissolve in 10 cc. of water in a separator. Add 1 cc. of a freshly prepared saturated solution of sodium bicarbonate and 25 cc. of ether and shake the mixture. After separation, draw off the lower layer into a second separator and transfer the ethereal layer to a third separator. Extract the mixture in the second separator repeatedly with 15 cc. portions of ether until the alkaloid has been completely removed, using the second and the first separators alternately for the shaking, and collecting all the ethereal solvent in the third separator. Discard the aqueous solution, and wash the ethereal solution of alkaloid three times with 5 cc. each of water. Unite the aqueous washings in a clean separator and extract the solution with a little fresh ether. Discard the aqueous portion, wash the solvent with water, discard the washings, and add the washed solvent to the main portion of the ethereal solution. Add 20 cc. of 0.02 *N* sulfuric acid to the ethereal solution of the alkaloid in the separator and shake the mixture thoroughly. Transfer the mixture to a beaker, wash the separator with two portions of 5 cc. each of water, adding the washings to the acid liquid in the beaker, and evaporate the ether without delay at a low temperature, preferably on the water bath by the aid of a blast of air. Titrate the excess of acid with 0.02 *N* sodium hydroxide, using 1 drop of methyl red test solution as indicator.

1 cc. of 0.02 *N* sulfuric acid = 0.00625 gram of apomorphine hydrochloride, $C_{17}H_{17}O_2N.HCl + \frac{1}{2} H_2O$.

METHOD II.—ASSAY FOR APOMORPHINE HYDROCHLORIDE USING CHLOROFORM AS SOLVENT.

Dissolve a number of tablets equivalent to about 0.065 gram of the alkaloid or its salt in 10 cc. of water in a separator. Add 1 cc. of a concentrated solution of sodium bicarbonate and 25 cc. of chloroform and shake the mixture. After separation, draw off the lower layer into a second separator. Repeat the extraction of the mixture in the first separator with 5 cc. portions of chloroform until the alkaloid has been completely removed. Unite the solvent and wash it three times with 5 cc. of water. Unite the washings and extract the solution with a little fresh chloroform. Discard the aqueous portion, wash the solvent with water, discard the washings, and add the solvent to the main portion. Add 20 cc. of 0.02 *N* sulfuric acid to the chloroform solution of alkaloid in the separator and shake the mixture thoroughly. Draw off the chloroform layer into a third separator, wash it twice with small portions of water, and add the aqueous washings to the acid liquid in the second separator. Titrate the excess of acid with 0.02 *N* sodium hydroxide, using 1 drop of methyl red test solution as indicator.

A mixture of 25 parts of apomorphine hydrochloride and 75 parts of milk sugar was prepared by the associate referee. A portion of the mixture and a copy of each of the methods of assay were forwarded to each of the collaborators. The associate referee also served as a collaborator. The collaborators were directed to use from 0.25 to 0.3 gram of the mixture in each assay. Results were received from the following collaborators:

E. O. Eaton, Food and Drug Inspection Station, San Francisco, Calif.
J. D. Hoskins, Zemmer Co., Pittsburgh, Pa.
Wm. D. Taylor, Schieffelin Co., New York.
Bernardelli and Weylard, Powers-Weightman-Rosengarten Co.
L. E. Warren.

The results obtained by the several collaborators are given in the accompanying table.

COMMENTS OF COLLABORATORS.

The comments were as follows:

Hoskins: In the ether method, after adding the ether solution to the standard acid and evaporating the ether from the combined solutions, we found the acid solution colored so that, upon titration, the end point was very indistinct. However, by using the chloroform method of washing the ether solution with the standard acid, and again washing the ether solution with three portions of water and adding to the standard acid and titrating without the necessity of evaporating the ether, we had no trouble with the acid solution being colored before the methyl red was added. With this modification it seems to us that the ether method is preferable, as it avoids transferring from one separator to another, as is necessary in the chloroform extraction.

Rosin: Both chemists expressed a preference for the ether method, first because there is less manipulation and second because the tendency for emulsification is less than with chloroform.

Warren: When the ether method is carried through without delay so as to avoid undue exposure to light and air the solution remains practically colorless and may be titrated with ease. However, if any of the alkaline solution is allowed to stand for more than a few minutes, discoloration will result and interfere with the determination

Results of collaborative assays of apomorphine hydrochloride.—Lactose mixtures.

COLLABORATOR	METHOD	TAKEN	0.02 *N* ACID CONSUMED	EQUIVALENT OF APOMORPHINE HYDROCHLORIDE		FOUND
		gram	*cc.*	*gram*	*per cent*	*per cent*
Eaton	E	0.3	11.85	0.0741	24.7	98.8
	E	0.3	11.97	0.0748	24.9	99.6
	C	0.3	11.96	0.0748	24.9	99.6
	C	0.3	11.96	0.0748	24.9	99.6
Hoskins	E	0.280	11.2	0.0700	25.0	100.0
	E	0.270	10.8	0.0650	25.0	100.0
	E	0.2655	10.66	0.0625	24.9	99.6
	E	0.269	10.8	0.0675	25.1	100.4
	E	0.305	12.1	0.0756	24.8	99.2
	C	0.263	10.6	0.0662	25.2	100.8
	C	0.253	10.1	0.0631	25.0	100.0
	C	0.275	11.0	0.0687	25.0	100.0
Bernardelli	E	0.3100	12.10	0.0756	24.4	97.6
	C	0.3420	13.10	0.0819	23.9	95.6
Weylard	E	0.3030	11.45	0.0716	23.7	94.8
	C	0.3004	11.45	0.0716	23.8	95.2
Taylor	E	0.2590			24.4	97.6
	E	0.2505			24.7	98.8
	C	0.2540			24.4	97.6
	C	0.2520			25.0	100.0
Warren	E	0.2902	11.72	0.0732	25.21	100.9
	E	0.3007	12.06	0.0754	25.11	100.4
	E	0.3008	12.00	0.0750	25.0	100.0
	C	0.3006	12.02	0.0751	25.0	100.0
	C	0.3002	12.02	0.0751	25.0	100.0

of the end point, as Hoskins has observed. In order to avoid delay I prefer to make but one assay at a time, that is, not to run duplicates simultaneously. I prefer the ether method for the reasons pointed out by Rosin's assistants.

Taylor: The method using ether as solvent is somewhat easier to handle as the separation of ether from the aqueous layer is more complete than that of chloroform. It was noted that the final acid solution of the alkaloid was colored a greenish color, while the ether layer was of a violet tint. Both colors darkened as the evaporation of the ether at a fairly low temperature was taking place. This color interfered somewhat in the titration as it masked the end point to some degree. The chloroform method did not give any objectionable color but was more tedious on account of the poor separation of the chloroform from the water. The end point in this method was very easily observed.

No report on santonin was given by the associate referee.

REPORT ON ETHER.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The Associate Referee on Ether found little opportunity to give attention to the subject during the past year, owing to unusual conditions that prevailed at times in his laboratory.

The method that will first be considered is proposed in an article, entitled "Ether and Alcohol—Quantitative Determination", by E. V. Somogyi[1].

RECOMMENDATION[2].

It is recommended that the work on the identification and estimation of ethyl ether in admixture be continued.

REPORT ON BIOASSAY OF DRUGS.

By E. W. SCHWARTZE[3] (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The bioassay method for the quantitative determination of mydriatics —atropine, hyoscyamine, scopolamine (hyoscine)—was developed in the Pharmacological Laboratory of the Bureau of Chemistry by J. C. Munch, for use in assaying drug samples where chemical assays were unsuited. The outlined method was submitted to several collaborators. Reports so far received have been satisfactory, but in some instances the method has not been given a rigorous trial. The associate referee is convinced that certain mydriatics can be assayed upon the cat's eye.

The method is as follows:

APPARATUS.

(**a**) One cc. Mohr pipets, graduated in 0.01 cc., with slender tips that deliver exactly 0.05 cc. per drop.

(**b**) 100-watt, nitrogen-filled, electric lamps, or equally intense illumination.

ANIMALS.

Adult cats in good physical condition, weighing over 1500 grams, and accustomed to being handled.

PREPARATION OF SAMPLE.

Dissolve a representative number of tablets, or a sufficient quantity of powder, in approximately neutral distilled water, to make a solution containing 1 mg. of the alkaloid per cc. of solution. If the alkaloids themselves are taken, add the equivalent quantities of acid to convert them into the corresponding salts. Add two drops of approximately 0.02 *N* acid per 50 cc. of solution.

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[1] *Z. angew. Chem.*, 1926, **39**: 280.

[2] For report of Sub-committee B and action of the association, see *This Journal*, 1926, **10**: 70.

[3] Present address: Mellon Institute, Pittsburgh, Pa.

For great accuracy, the results of chemical assay upon the sample should be followed in the preparation of solutions; when such accuracy is unnecessary, the declaration of strength on the label may be accepted as the basis for the preparation of the solution.

DETERMINATION OF CAT'S THRESHOLD.

Place a cat about one foot from a 100-watt electric lamp, and determine the maximum contractility of its pupils under this condition. Drop 0.05 cc. of the freshly prepared standard mydriatic solution, obtained by diluting the 1 mg.-per-cc. solution, into the outer margin of one eye, leaving the other eye untreated as a control. Compress the inner canthus, while opening and closing the lids, until the fluid has apparently disappeared (10 to 30 seconds). Return cat to cage.

One and two hours after application (for atropine, 3 and 4 hours also), place cat under the same conditions, and note any differences in diameter between the pupils of the treated and the untreated eyes. (A satisfactory reaction is produced when the pupil of the treated eye is just perceptibly wider (0.5 to 1.0 mm.) than the pupil of the untreated eye.) Do not use the same eye for another assay for at least 24 hours.

If the concentrations given by Munch fail to produce a satisfactory reaction, repeat the test with a stronger or weaker solution until the minimum effective concentration is found. (This concentration may vary somewhat for different cats, but it is essentially constant for the same cat.)

BIOASSAY OF UNKNOWN SOLUTIONS.

Dilute the 1 mg.-per-cc. solution to be tested to the minimum effective concentration for the cats to be used, and drop 0.05 cc. of this dilution into one eye of the cat, following the same procedure as in the determination of the minimum effective concentration. Also prepare stronger and weaker solutions, and apply to one eye of each of the other cats used. Test various concentrations until one is obtained that produces satisfactory mydriasis of the same degree as the standard solution when tested on two or more cats.

To obtain the milligrams of alkaloid present in each cc. of the original solution, multiply the milligrams per cc. found to be the cat's minimum effective concentration by the dilution employed. Knowing that the original solution was made to contain 1 mg. of alkaloid per cc., calculate the quantity of mydriatic present, and express as percentage of the total alkaloid.

The accuracy attained when this method is used will depend upon the care taken to standardize the cats before attempting the assay of unknown solutions. The actual work with the unknown is a very small part of the total labor.

The method recommends 0.05 cc. for physical reasons; for physiological reasons, it might be more satisfactory to reduce this quantity to 0.04 cc. or even 0.03 cc.

The associate referee is unwilling to rely upon the constancy of volume of drops, even from a standardized pipet. He has difficulty in watching the reading and the cat's eye simultaneously. It is therefore recommended that special pipets or syringes be used to deliver only the exact quantity wanted.

The associate referee's criterion for determining the effect of a drug is the maximum constriction of the pupil. Enlargement of one pupil (using the other as a control) may be observed if this procedure is not strictly followed; whereas at the maximum constriction, no difference is noted. The criterion chosen is arbitrary, but the soundness of selection is based upon the logical assumption that at the maximum constriction the cat is using every muscle fiber in the circular muscle of the iris. This condition is more easily reproduced than any intermediate states of non-maximal constriction, in which the number of active fibers is unknown.

REPORTS AND COMMENTS OF COLLABORATORS.

J. C. Munch: This method was developed primarily for mixtures, such as morphine and atropine. Over a hundred solutions have been treated by it. By careful attention to details, it has been possible to differentiate between solutions of mydriatic alkaloids varying by 10 per cent in concentration.

A solution containing 4.0 mg. of hyoscyamine per liter was readily distinguished from others containing 3 6 mg. and 4.4 mg. per liter, the concentrations of all solutions being unknown at the time the tests were made. Bioassay of an atropine-morphine mixture made to contain 83 mg. of atropine per liter gave results of 100 mg. per liter. Several solutions containing atropine, hyoscyamine, or hyoscine were tested with similar agreements with chemical assay. Solutions of morphine and atropine sulfate, and hypodermic tablets of the same composition, have been assayed, and agreement was found within the limits of accuracy of the method with chemical assays.

The method cannot replace the chemical assay, of course. However, in the assay of morphine-atropine, hyoscine-morphine-cactin, atropine-papaverine, and similar mixtures, it seems to have a definite place. In studying the racemization of hyoscyamine to atropine, it has proved very useful.

I have found the following concentrations statistically to be average minimum effective concentrations:

	mg per liter
Atropine	12
Hyoscyamine	4
Scopolamine	0.4
Homatropine	100
Cocaine	100
Euphthalmin	50,000

Data upon the first three were more carefully worked out than on the others.

L. W. Rowe, Parke-Davis Co.: In these experiments the threshold was not so definitely uniform as it was found to be in your work. The accuracy of the method certainly does not seem to be within 10 per cent although unknowns were not tested, and hyoscyamine may be more definite in action than atropine. There are certainly possibilities in the method when mixtures of mydriatic alkaloids are concerned, but with atropine the quantitative possibilities do not seem to be within the 10 per cent * * *. We were surprised at the time factor being so variable in different animals even with the same dose. It would seem that with such a definite technique the time of maximum action should be much more uniform and also much shorter after instillation of the drug.

Carl Nielsen, Abbott Laboratories: * * * The few tests we have conducted with it have indicated that the method offers a simple and practical quantitative assay for mydriatics in compound mixtures * * *. We welcome this valuable method of assay and intend to apply it as a routine test for quantitative assay of mydriatics in mixtures of this character.

H. R. Watkins, Bureau of Chemistry: I used the method developed in the Pharmacological Laboratory and obtained satisfactory results on atropine and hyoscyamine—the hyoscine experiments were not completed. The atropine and hyoscyamine thresholds were found to be the same as described in the method of assay.

It was found that individual cats varied, hence it was necessary to standardize the cats and to know each cat. The use of standardized pipets graduated in 0.01 cc. was satisfactory.

RECOMMENDATIONS[1].

It is recommended—

(1) That the method presented be adopted as a tentative method.

(2) That the application of the method to the quantitative bioassay of other mydriatics be studied during the coming year.

REPORT OF COMMITTEE ON CONSTITUTION AND BY-LAWS.

At its last meeting the association adopted a resolution directing that any proposed amendments to the constitution and by-laws of the association be published in *The Journal* in advance of the date of the next convention, in order that members of the association might have the opportunity of giving such amendments due consideration before final action upon the question of their adoption.

The tentative revised draft of the constitution and by-laws presented herewith was prepared by W. W. Skinner, W. H. MacIntire, C. A. Browne, and O. Schreiner, who gave much time and thought to the task delegated to them.

The chairman was unable to be in Washington at the time and to take part in the work of the revision, but he concurs with the other members and collaborators in the report submitted and desires to express his appreciation of the valuable service rendered by them.

B. B. Ross,
Chairman of Committee on Revision of
Constitution and By-Laws.

CONSTITUTION AND BY-LAWS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS[2].

CONSTITUTION.

Article I.

Name and Object.

This association shall be known as the Association of Official Agricultural Chemists of North America.

The objects of the association shall be—

(1) To secure, devise, test, and adopt uniform and accurate methods for the analysis of fertilizers, soils, foods, feeding stuffs, dairy products, medicinal products, and other materials relating to agricultural pursuits;

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[1] For report of Sub-committee B and action of the association on Bioassay of Drugs, see *This Journal*, 1927, **10**: 70.

[2] See *This Journal*, 1920, **3**: 586-9, for present constitution.

(2) To secure uniformity in the statement of analytical results;

(3) To conduct, promote, and encourage research in chemistry in its relation to agriculture; and

(4) To afford opportunity for the discussion of matters of interest to agricultural chemists.

ARTICLE II.

Membership.

ACTIVE MEMBERS.

Chemists connected with the following agricultural institutions shall alone be eligible *ex officio* to active membership:

(1) The United States Department of Agriculture;

(2) Any national, state, or provincial experiment station, college, or body engaged in research in agricultural chemistry; and

(3) Any national, state, or provincial institution or body charged with official control of any of the materials named in Article I.

ASSOCIATE MEMBERS.

Chemists connected with municipal laboratories charged with control of any of the materials or subjects named in Article I are eligible *ex officio* to associate membership. Chemists engaged in research in agricultural chemistry who are not eligible to active membership and active members of the association who lose their right to such membership by retiring from the positions indicated above as requisite for eligibility to active membership may be elected to associate membership upon recommendation of the Executive Committee.

HONORARY MEMBERS.

Upon recommendation of the Executive Committee, persons may be elected to honorary membership by the two-thirds vote of those present at any regular meeting of the association.

ARTICLE III.

Officers and Committees.

The officers of the association shall consist of a president, a vice-president, and a secretary who shall also act as treasurer.

These officers shall be elected annually from and by the active members and they shall perform the usual duties of their respective positions. These officers, together with two other active members to be elected by the association, shall constitute the Executive Committee. The special duties of the officers of the association shall be further defined, when necessary, by the Executive Committee. Upon the recommendation of the Executive Committee, the president shall appoint a chairman and a committee of nine other members, which shall be designated as a Committee on Recommendations of Referees, one-third of the membership of which shall be appointed at intervals of two years to serve six years, the chairman to be appointed annually. The chairman shall divide the nine members into subcommittees (A, B, and C) and shall assign to each subcommittee the reports and subjects which it shall consider. At the annual meeting, upon the recommendation of the Committee on Recommendations of Referees, the president shall appoint from among the active or associate members of the association, a referee and associate referees for each of the subjects to be considered by the association. It shall be the duty of these referees—

(1) To prepare and distribute samples and standard reagents to members of the association and others desiring the same;

(2) To furnish blanks for tabulating analyses;

(3) To present at the annual meeting the results of the work done and recommendations for methods to be based thereon; and

(4) To direct and encourage general discussion at the meetings.

ARTICLE IV.

Meetings.

The annual meeting of the association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the Executive Committee. Announcement thereof shall be made, if possible, three months prior to the time of said meeting. Special meetings shall be called by the Executive Committee when in its judgment it may be necessary.

ARTICLE V.

Changes in Constitution.

All proposed changes or amendments to this constitution shall be presented in writing and read in full to the association not later than the second day of the regular annual meeting, shall be referred to the Executive Committee, and after a report from this committee may be adopted as the first order of business on the third day by a vote of three-fourths of the active members present.

BY-LAWS.

(1) Any amendment to these by-laws or changes therein may be proposed in the same manner and adopted by the same procedure as amendments to the constitution, but only a two-thirds vote of the active members present shall be required for their adoption.

(2) These by-laws or any portion of them may be suspended at any regular meeting of the association without previous notice by a vote of three-fourths of the active members present.

(3) Only one qualified active member of a college, experiment station, bureau, board, or other institution shall be entitled to vote on general questions before the whole association. At the discretion of the Chair, any institutional vote upon which there does not seem to be adequate representation may be conducted by letter ballot.

(4) In voting upon questions involving methods of analysis, definitions, nomenclature, and laws or regulations relating to materials mentioned in Article I of the Constitution, each of the said institutions shall be entitled to vote only upon questions relating to those materials over which said institution exercises official control.

(5) A method shall not be adopted as official or an official method be amended until such method or amendment has been recommended as official by the appropriate referee at two annual meetings.

(6) No changes shall be made in the methods of analysis used in official inspection until an opportunity shall have been given all active members having charge of the particular inspection affected to test the proposed changes.

(7) A method shall not be adopted as tentative or a tentative method amended until such method or amendment has been reported by the appropriate referee and published in the proceedings of the association.

(8) When any officer ceases to be eligible for membership in the association, his position shall be considered vacant, and a successor may be appointed by the Executive Committee to continue in office until the next regular meeting. Should any referee or

associate referee resign or cease to be eligible for membership in the association, his office shall be considered vacant and a successor shall be appointed as prescribed in Article III of the Constitution. Should a vacancy occur in the Executive Committee, such vacancy may be filled by the action of the other members.

(9) Chemists connected with commercial firms or institutions and others interested in the objects of the association who are not eligible to either active or associate membership may attend its meetings, take part in its discussions, and, if permission is secured from the Executive Committee, may present papers.

(10) Each college, experiment station, bureau, board, or other institution entitled to representation in the association shall contribute annually $5.00 prior to the first of January following the regular annual meetings.

(11)[1] A Board of Editors of *The Journal* of the association, consisting of five members, one of whom shall be designated the chairman, shall be appointed by the president with the advice and consent of the Executive Committee. These members shall serve for a period of five years, a new member being appointed each year.

(12)[1] A fertilizer definition or interpretation shall not be adopted as tentative or a tentative definition or interpretation amended until such definition or interpretation has been recommended by the Committee on Definitions of Terms and Interpretation of Results on Fertilizers and published in the proceedings of the association.

(13)[1] A fertilizer definition or interpretation shall not be adopted as official or an official definition or interpretation be amended until such definition or interpretation has been recommended by the Committee on Definitions of Terms and Interpretation of Results on Fertilizers at two annual meetings.

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[1] Added at the 1922 convention.

CONTRIBUTED PAPERS.

AN INEXPENSIVE AND ACCURATE GAS CHAIN FOR LIQUIDS LIGHTER THAN SATURATED POTASSIUM CHLORIDE SOLUTION.

By HENRY C. WATERMAN (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.).

In the majority of the simpler forms of hydrogen electrode vessel now on the market, the liquid junction is made by the dipping of a tube carrying the bridge solution into the liquid under examination. This arrangement causes more or less rapid diffusion of small quantities of potassium chloride into the space about the electrode, and so tends to produce low results. There are now available many ingenious devices by which this difficulty is overcome, and by which, also, there is secured the important advantage of a sharp liquid junction. None of these devices, however, so far as the writer is aware, can be either made or repaired in the laboratory by the chemist of ordinary glass-blowing skill, and all of them are expensive to purchase outright. An electrode apparatus embodying the accuracy features of the more elaborate types in a design simple enough to be set up quickly, with little or no glass blowing and at small expense, remains, therefore, an important desideratum. It was to meet such a need that the design presented in this note was devised.

In this laboratory the apparatus has given uniformly satisfactory results with solutions of a wide variety, both in general composition and in pH value. (F. Hillig, for instance, has successfully used Form I of the new vessel for between 200 and 300 pH measurements on malt infusion-milk mixtures. Indeed, Form I (for foaming liquids) was suggested to the writer by this need, the mixtures foaming so badly that special provision was necessary.) The contamination of the sample solution with potassium chloride, mentioned previously, has not occurred in any experience with the apparatus; indeed, the connection between the test solution and the saturated potassium chloride bridge has been left open for a period of 18 hours without the appearance of chloride in the body of the electrode vessel. The exclusion of carbon dioxide and other atmospheric contaminants appears to be sufficiently provided for, since the results with such standard checking solutions as 0.05 M potassium hydrogen phthalate have shown no error greater than a few thousandths pH, except when the error could be traced to the use of impure potassium chloride in either the bridge or the calomel electrode. A sharp liquid junction, easily and quickly reproducible with very small loss of the experimental material, is readily obtained;

foaming liquids and those containing sediments are handled without undue complication of the manipulation and without contamination of the salt bridge with sediment. (The apparatus is adapted, therefore, for quinhydrone as well as for bubbling hydrogen.) The volume of sample solution required is comparatively small, 20 cc. being ample for duplicate determinations; the electrode vessel is easily removed and cleaned; and, finally, the entire assembly, with the exception of the sealing of a small platinum wire into the drawn-out end of a piece of half-inch tubing, to form the calomel electrode vessel, can be set up, in one of its two forms, without any glass blowing. It is hoped that this apparatus may fill the need for accurate, laboratory-made equipment until a better has been devised.

CONSTRUCTION.

Two forms of the electrode vessel proper are shown in the drawing. That shown at the right (Form II), in its place in the assembly, has been found most convenient for ordinary use. It may be made from a heavy-walled test tube having an internal diameter of about 20 mm., to the bottom of which is sealed an ordinary glass stopcock carrying tubing of about 5 or 6 mm. diameter. Form I, especially adapted for foaming liquids, is drawn to a much larger scale than that of the complete assembly. It consists of a stock size Squibb separatory funnel, converted to the present use without any glass blowing, by cutting the bevelled tip from the stem and rounding the cut edges slightly in a flame.

At the right are also shown the remaining parts of the gas chain. These will be seen to consist essentially of four T-tubes, short lengths of rubber tubing, and a separatory funnel to serve as a reservoir for saturated potassium chloride, together with a very simple form of saturated calomel electrode. The entire assembly—two hydrogen electrode vessels with their reservoirs and a common calomel electrode for the two—can be mounted on one medium sized ring stand, or the vessels and half-cell can be placed on one small sized stand and the reservoirs on another, as shown in Fig. 2. The construction is believed to be so plainly shown in the drawing and photograph that it will not be given in detail. It may be noted, however, that the tubing of T-1 should have an inside diameter about 2 mm. greater than the outside diameter of the electrode vessel stem. The writer's own set-up has approximately the following dimensions at the point mentioned, T-1 (vertical portion): outside diameter, 10 mm.; inside, 9 mm.; stem, outside diameter 7 mm., inside 5 mm. The remaining details, with the exception of the position of the reservoir, which is described under manipulation, may be obtained with sufficient accuracy from the drawing. The form of calomel half-cell shown was chosen not only for its easy and inexpensive construction, but also for its freedom from the annoyances incident to the dipping connection in a side-tube filled with mercury.

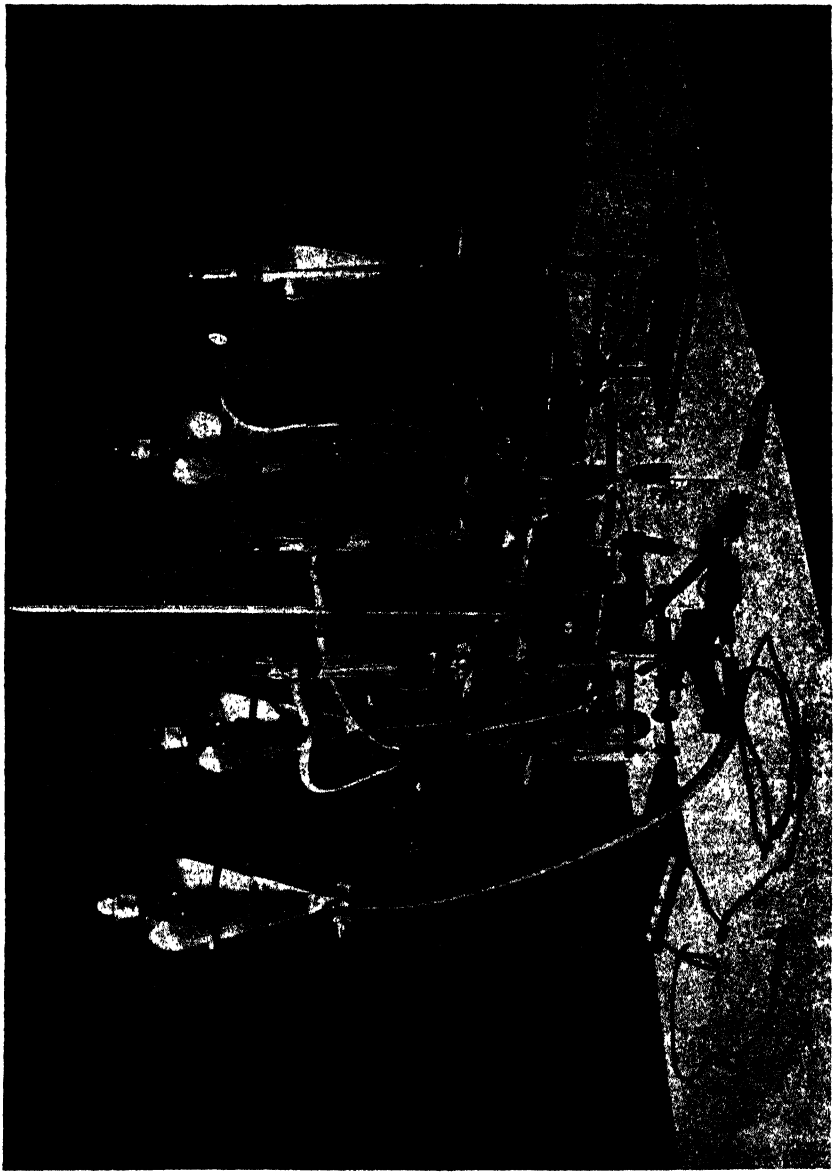
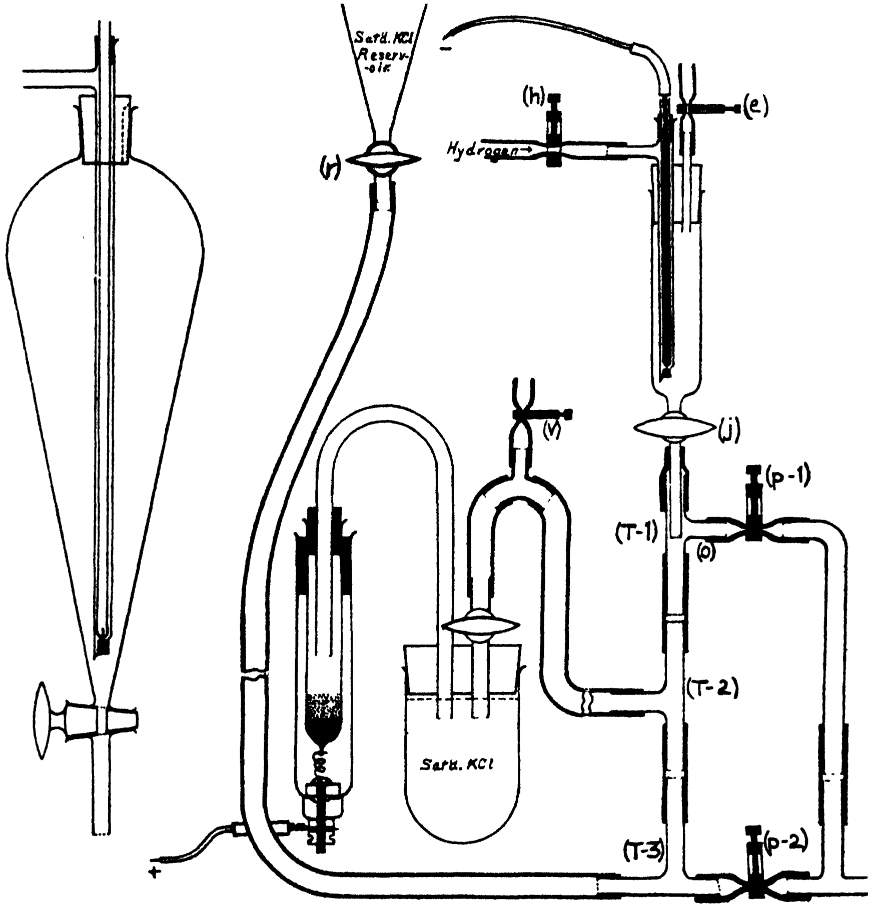


Fig. 2.



FORM I.   FIG 1.   FORM II

MANIPULATION.

After withdrawing the vessel proper from the rest of the assembly, wash and rinse it with distilled water, and dry it by wiping and by drawing or blowing through a current of air. Dip the end of the stem into the liquid to be examined, and fill the vessel to a height of 20 to 25 mm. by suction. (This ensures a bubble-free filling of the stem.) Rinse the electrode, previously plated and rinsed with distilled water, with about 0.5 cc. of the solution to be tested, and put it in place in the hydrogen jacket tube. Open the cock (r) momentarily, so that the bridge solution comes to the top of the upper outlet (o), then return the vessel proper to its position in the apparatus. Attach the hydrogen supply and allow the gas to bubble through at a suitable rate until equilibrium is attained.

To make a reading, open the pinchcock (p-1) of the upper outlet (o) for a moment, to release the pressure caused by the insertion of the electrode vessel stem; open the liquid-junction cock (j), keeping (p-1) closed; now reopen (p-1) for an instant, permitting but two or three drops of the sample solution to escape into the surrounding saturated potassium chloride solution. Close (j), open (r), and once more open (p-1) *slightly*, allowing 1 or 2 cc. of the bridge solution to sweep out the mixture, potassium chloride and sample solution, from about the tip of the electrode vessel stem, which at that point forms a sharp junction. Finally, close first (p-1) and then (r), and open (j).

The residual pressure in the tubing from the potassium chloride reservoir to the gas chain raises the junction about 5 or 6 mm. in the electrode vessel stem. If this action does not occur, or if the junction rises too high in the stem, move the reservoir up or down until the correct adjustment is obtained. Having taken the reading, close (j) until it is again desired to check the progress of the determination. When the potential difference ceases to rise, make a new junction as just described; shut off the hydrogen; close the pinchcock (h); pinch and release the tubing between (h) and the vessel to expel the hydrogen from the end of the jacket tube and draw the sample solution well up about the electrode; close (e); and take the final reading. The junction may be renewed every time a check reading is taken and requires only about 15 seconds, but in the experience of the writer the original junction remains sharp enough for all save final readings.

The manipulation requires a lengthy statement in words, but in practice it is very quickly and easily performed.

Sediments. If any sediment from the sample settles into the potassium chloride column in (T-1), (T-2), and (T-3), remove the vessel at the end of the determination and open (p-2), dropping the entire column into the waste line. Close (p-2), refill by opening (r), and repeat the draining if necessary. In draining the column, if a bubble of air enters the arch of tubing between T-2 and the potassium chloride trap, it may be removed by opening the vent (V). This vent is also useful for ridding the bridge of air bubbles in setting up the gas chain.

Foaming Liquids. Use Form I of the electrode vessel. As this form can be made without glass blowing, it might well be used for all determinations; no greater volume of sample is required than for the tubular form. Despite the obvious theoretical objection to the large space above the solution, it has been found that the results with the Squibb funnel form of the vessel are as accurate as those obtained with the tubular form.

The manipulation of the funnel form differs from that of the tubular in one point only. The necks of the smaller Squibb funnels do not admit a stopper large enough to carry both the hydrogen jacket tube and an outlet tube with cock. The hydrogen outlet, therefore, takes the form of a slot cut in the side of the stopper. This slot is not extended to the top of the stopper, but it is made of such a height that when the stopper is firmly, but not fully, inserted, the slot is open. When the hydrogen is shut off for the final reading, however, the stopper is fully inserted, thus preventing either entrance of air or loss of the hydrogen atmosphere.

Accuracy. 0.05 *M* solutions of potassium hydrogen phthalate in carbon-dioxide-free distilled water were used as checking standards, at various temperatures within the range of 20°–30°C. The theoretical value is pH 3.97 for all temperatures within the range stated[1]. All the results obtained in this laboratory have been between pH 3.96 and pH 3.97, most of them between pH 3.965 and pH 3.970. Results with the tubular and funnel forms of the apparatus are the same.

Manipulation time. Five minutes is required to remove, wash, dry, fill, and replace the electrode vessel, and prepare the electrode. Forming or renewing junctions has already been stated to require about 15 seconds. Equilibrium is attained at the same rate as with other bubbling types of electrode vessel.

A similar scheme for securing sharp liquid junction has been used by F. A. Elliott[2] and by Elliott and Acree[3]. Their designs, however, are among the most expensive of electrode vessels because of the complicated and delicate glass work; and, furthermore, the electrode vessels proper are made in one piece with the potassium chloride bridge. It is impossible, therefore, to remove, clean, and dry the electrode vessel proper for each determination. The convenience and economy of sample solution obtained by the separable construction have already been noted.

SUMMARY.

A simple design of sharp-junction hydrogen electrode vessel of the bubbling type is described, one form of which can be set up without any glass blowing from materials available in almost any laboratory. The vessel possesses the advantages of low cost, rapid manipulation, freedom from potassium chloride contamination of the solution under examination, exclusion of atmospheric carbon dioxide, small volume of sample, and accuracy. Results with 0.05 *M* potassium hydrogen phthalate as test solution show uniformly less than 0.01 pH unit variation from the theoretical value for this solution as given by Clark.

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[1] Clark, W. M. The Determination of Hydrogen Ions, 2nd ed., p. 274, Williams and Wilkins Co., 1925.
[2] Bull. 100. The Will Corporation, Rochester, N. Y.
[3] Pyroelectr. Bi-monthly Bull., No. 14, September, 1921.

THE ESTIMATION OF MILK FAT IN MILK CHOCOLATE BY MEANS OF A MODIFIED XYLENE NUMBER.

By C. A. Greenleaf[1].

INTRODUCTION.

The usual method of estimating milk fat in milk chocolate, and that adopted tentatively by the Association of Official Agricultural Chemists[2], employs the Reichert-Meissl number of the extracted fat as the basis for calculation. Recent collaborative work reported by nine analysts for that association, however, showed a variation between results on the same sample of about 36 per cent of the total quantity of milk fat present[3]. Since the Reichert-Meissl procedure is quite rigidly empirical and the results are strongly influenced by variations in experimental conditions, these results are not surprising. In view of this variation, however, it seemed desirable to make further study of methods for estimating milk fat in such mixtures, especially methods from which it might be possible to eliminate the variable factors.

A conspicuous characteristic of milk fat is its content of combined butyric acid, and this fact has been utilized widely in efforts to determine milk fat in the presence of other fats, such as coconut oil and palm kernel oil, which contain considerable quantities of volatile fatty acids other than butyric. While the interfering influence of these fats was not anticipated in the work described in this paper, the methods referred to suggested means of controlling experimental conditions more closely.

The method proposed by Kirschner[4] determines the number of cc. of $0.1N$ alkali required to neutralize an aliquot part of those Reichert-Meissl acids whose silver salts are soluble in neutral water solution. Formulas for the use of this value are given by Kirschner and also by Elsdon and Smith[5]. The principal objection to the method is that it is carried out on the distillate obtained in the Reichert-Meissl procedure, and is, therefore, subject to the uncertainties of the latter. The "A" and "B" number procedure of Bertram, Bos, and Verhagen[6] avoids this difficulty by removing the higher fatty acids as insoluble magnesium soaps and applying the silver-soap separation previous to the distillation of the volatile fatty acids. As a result of these separations, the fatty acid remaining for distillation is principally butyric, 96-97 per cent of which distils under the conditions of the procedure, regardless of reasonable variations in the rate of distillation.

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[1] Under assignment to the Food Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C., from the U. S. Veterans' Bureau.

[2] *Methods of Analysis, A. O. A. C.*, 1925, 345.

[3] *This Journal*, 1926, **9**: 465.

[4] *Z. Nahr. Genussm.*, 1905, **9**: 65.

[5] *Analyst*, 1925, **50**: 53.

[6] *Z. deut. Öl-Fett-Ind.*, 1924, **44**: 445, 459.

Other methods for approximating the content of butyric acid, such as the "Gilmour Number"[1] and the "Butyric Acid Number" of Kuhlmann and Grossfeld[2], depending on partition between an aqueous layer and a layer of insoluble fatty acids, were not studied in this investigation on account of the obvious difficulty of fixing the composition of the fatty acid phase.

EXPERIMENTAL.

In order to study the method of Bertram, Bos, and Verhagen, a series of mixtures of cocoa butter and butterfat was made up and examined by that procedure, as modified by Kuhlmann and Grossfeld[3], and further modified by the writer to use 10 grams of the fat and proportionate quantities of the reagents. This change was made because only the "B" number was to be determined, the "A" number being related to the detection of coconut or palm kernel oil.

In the procedure followed, 10 grams of the fat was saponified with a glycerin-potassium hydroxide mixture, diluted, and treated with 51 cc.

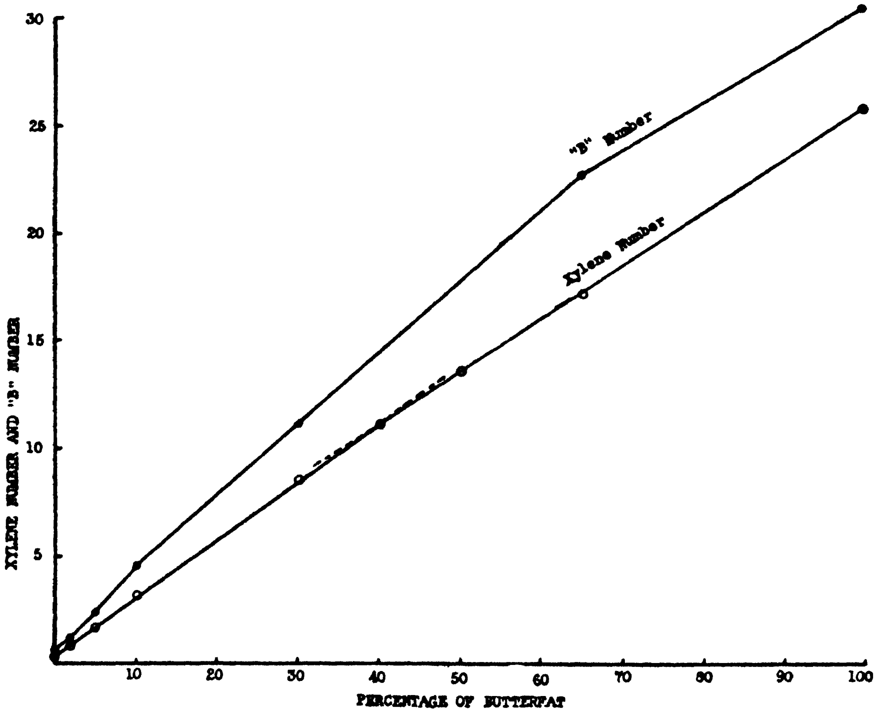

FIGURE I

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[1] *Analyst*, 1925, 50: 272.
[2] *Z. Untersuch. Lebensm.*, 1926, 51: 31.
[3] *Z. angew. Chem.*, 1926, 39: 24.

of hot 15 per cent magnesium sulfate solution at 80°C. After cooling to 20°C. and making up to 260 grams, the mixture was filtered. Then 200 cc. of the filtrate was neutralized with 0.5*N* sulfuric acid, made up to 250cc., treated with 2 grams of powdered silver sulfate, and filtered. Of this second filtrate, 200 cc. was acidified with 50 cc. of dilute sulfuric acid (13 cc. of concentrated sulfuric acid to 500 cc.) and distilled, 200 cc. of distillate being collected. The number of cc. of 0.1*N* alkali required to neutralize the distillate, minus the blank from a similar determination conducted without fat, is the "B" number. The "B" number corresponds to 6.4 grams of the fat, and is a measure of the volatile fatty acids whose magnesium and silver salts are soluble.

The results are shown in Table 1. Although close duplication was obtained and the percentages found show fair agreement with the actual, when the results are plotted (Figure 1, upper line), it is evident that no simple mathematical relation exists between the "B" number and the percentage of butterfat.

When the butterfat is diluted with other fat, irregularities are apparently introduced through the partial solubility of the silver salts of the volatile acids other than butyric acid that are present in butterfat. The influence of caproic acid in particular was shown by the following experiment:

TABLE 1.

Estimation of butterfat from the "B" number.

BUTTERFAT	"B" NUMBER	BUTTERFAT FOUND
per cent		*per cent*
0	0.65	0
2	1.22 1.22	2.0 2.0
5	2.48 2.48	4.4 4.4
10	4.6 4.5	10.1 10.0
30	11.40 11.25	28.1 27.8
65	22.70 22.60	62.0 61.7
100	30.8 30.5 30.0	90.0 89.0 87.5

Figures under "Butterfat Found" were obtained from the chart prepared by B., B. and V., to which reference has been made.

A quantity of caproic acid (0.195 grams), an excess over the amount to be expected in the quantity of butterfat used in the "B" number procedure, was neutralized with sodium hydroxide, diluted to 250 cc., treated with silver sulfate, as in the "B" method, and filtered. Using this filtrate, the "B" number procedure was completed from this point as previously described. The distillate required 2.35 cc. of 0.1*N* sodium hydroxide, blank 0.20, net 2.15 cc. of 0.1*N* sodium hydroxide.

As much as 2.15 cc., then, of the "B" number may be due to caproic acid not removed by the silver separation. If the percentage of butterfat present is high enough to yield an excess of caproic acid, this effect is constant, the excess of caproic acid being precipitated. Low percentages do not exceed the solubility of silver caproate, and all the caproic acid remains in solution, its effect on the "B" number then being proportionately greater. Since butterfat contains, in addition to butyric and caproic acids, caprylic and capric, the silver salts of which are not entirely insoluble, the relation between the "B" number and the content of butterfat could be expected to vary through the entire range, as the results show that it does.

Van Raalte[1] found that when dilute aqueous solutions of butyric and caproic acids are shaken with 20 per cent of their volume of xylene, about 79 per cent of the caproic acid is removed and only about 6 per cent of the butyric, and that under the same conditions caprylic acid and higher homologs are removed practically entirely by the xylene. His "xylol number" procedure[2] is based on this separation. In this procedure 5 grams of fat is used and the Reichert-Meissl number is determined by the Leffman and Beam method. To the titrated distillate is added the equivalent amount of 0.1*N* sulfuric acid, and 110 cc. is shaken with 22 cc. of xylene. After standing, the aqueous layer is filtered, and 100 cc. is titrated with 0.1*N* alkali, the result being calculated back to 5 grams of fat. Using this value and the Reichert-Meissl number, Van Raalte also gives formulas and tables for calculating the percentage of butterfat in mixtures. This procedure is subject to the same criticism as that of Kirschner's—it is carried out on the Reichert-Meissl distillate. In an attempt to overcome this difficulty a new procedure was developed by the writer embodying the removal of higher fatty acids as insoluble magnesium soaps and extraction with xylene previous to distillation of the volatile acids. The details are as follows:

MODIFIED XYLENE NUMBER.

REAGENTS.

(a) *Glycerin—potassium hydroxide mixture.*—To 300 cc. of C. P. glycerin, add 80 cc. of potassium hydroxide solution containing 750 grams per liter.

(b) *Magnesium sulfate solution.*—150 grams per liter.

(c) *Dilute sulfuric acid.*—17 cc. of concentrated sulfuric acid diluted to 500 cc.

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[1] *Chem. Weekblad.*, 1926, **23**: 222.

[2] *Ibid.*, 1927, **24**: 59.

PROCEDURE.

Into a tared 500 cc. Erlenmeyer flask weigh exactly 10 grams of the fat. Conduct also a blank determination without fat. Add 20 cc. of glycerin-potassium-hydroxide mixture and saponify gently over a small flame until the solution is perfectly clear, avoiding overheating. Add 175 cc. of hot, recently boiled distilled water, place in a water bath, and bring the temperature to 80°C. Add gradually 51 cc. of the hot magnesium sulfate solution, measured at 80°C., whirling the contents of the flask. Return to the bath and hold at 80° for 5 minutes with frequent shaking. Cool with shaking to 20°C., bring the contents of the flask to 260 grams with distilled water at 20°C., stopper, and shake vigorously. Place in a water bath at 20°C. for 5 minutes.

Filter at once on a Büchner funnel, using suction, pressing down the cake of magnesium soaps and draining thoroughly, in order to secure the necessary volume of filtrate. Transfer 200 cc. of the filtrate to a separatory funnel, pipet in 50 cc. of the dilute sulfuric acid, add 50 cc. of xylene, and shake thoroughly at intervals for 5 minutes.

Allow the layers to separate and draw off the aqueous layer through a folded filter. Transfer 200 cc. of this solution to a 500 cc. Florence flask, add 50 cc. of water and a few pieces of pumice, and distil exactly 200 cc. in about 40 minutes, using the Polenske apparatus. Titrate the distillate with 0.05 *N* alkali, using phenolphthalein. Subtract the blank and divide the remainder by 2 to obtain the "xylene number".

The same series of fat mixtures mentioned previously, with the addition of mixtures containing 40 per cent and 50 per cent butterfat, was examined by this procedure. The results are given in Table 2.

TABLE 2.

Estimation of butterfat from the modified xylene number.

BUTTERFAT	MODIFIED XYLENE NO.	CALCULATED BUTTERFAT
per cent		*per cent*
0	0.40	....
0	0.40	...
2	0.96	2.07
2	0.93	1.96
5	1.75	5.00
5	1.75	5.00
10	3.20	10.35
30	8.57	30.20
30	8.55	30.13
40	11.22	....
50	13.76	50.39
65	17.40	65.26
65	17.10	64.03
100	26.0	....
	25.9	....
	25.8	....

When plotted, these values give the points shown in Figure 1, lower line. While the values do not yield a single linear equation, the entire series can be approximated by two straight lines, one for values from 0 per cent to 40 per cent, the other from 40 per cent to 100 per cent butterfat, derived by the ordinary two-point formula:

$$\frac{P - P_1}{P_2 - P_1} = \frac{N - N_1}{N_2 - N_1},$$

where P = percentage of butterfat,
and N = xylene number.

From this relation, the line for values up to 40 per cent is:

$$\frac{P}{40} = \frac{N - 0.40}{11.22 - 0.40},$$

or

$$P = \frac{N - 0.40}{0.2705}, \text{ if } N \text{ is less than } 11.22 \text{ (1).}$$

From 40 per cent to 100 per cent,

$$\frac{P - 40}{100 - 40} = \frac{N - 11.22}{25.9 - 11.22},$$

or

$$P = \frac{N - 1.43}{0.2447}, \text{ if } N \text{ is greater than } 11.22 \text{ (2).}$$

These equations are shown plotted in Figure 1, lower graph, the two lines intersecting at the point corresponding to 40 per cent butterfat, having a xylene number of 11.22. The percentages obtained from the remaining xylene numbers by these equations, as shown under "Calculated Butterfat, per cent", in Table 2, show close agreement with the actual. Since the values for 0 per cent, 40 per cent, and 100 per cent were used in deriving the equations, they are not repeated under "Calculated Butterfat".

In order to base these equations on average values for cocoa butter and butterfat, additional samples of cocoa butter, butterfat, and mixtures of the two were examined by the above procedure. The complete results are given in Table 3.

Using the average values given in Table 3 for pure cocoa butter, pure butterfat, and mixtures containing 40 per cent butterfat, the equation for mixtures from 0 to 40 per cent butterfat is derived as above.

$$\frac{P}{40} = \frac{N - 0.19}{11.72 - 0.19},$$

or

$$P = \frac{N - 0.19}{0.288}, \text{ when } N \text{ is less than } 11.72 \text{ (3);}$$

and similarly for mixtures from 40 per cent to 100 per cent butterfat,

TABLE 3.

Modified xylene numbers of pure cocoa butters, butterfats, and mixtures of the two.

COCOA BUTTER MODIFIED XYLENE NO.		BUTTERFAT MODIFIED XYLENE NO.		MIXTURES, 40 PER CENT BUTTERFAT, 60 PER CENT COCOA BUTTER MODIFIED XYLENE NO.*	
I	0.40 0.40	I	26.0 25.9 25.8	I	11.09
II	0.30	II	25.5 25.5	II	11.14
III	0.15 0.15	III	25.80 25.55	III	12.04
IV	0.10 0.10	IV	27.50 27.35	IV	12.37
V	0.10 0.10	V	26.15 26.05	V	11.82
VI	0.10 0.10	VI	25.60 25.55	VI	11.69
Ave.	0.19	VII	25.40 25.20	VII	11.58
Max.	0.40	VIII	25.95	VIII	11.37
Min.	0.10	IX	27.45 27.30	IX	11.99
		X	26.45 26.25	X	11.67
		XI	26.25 26.10	XI	11.64
		XII	26.80 26.70	XII	12.14
		Ave.	26.18	Ave.	11.72
		Max.	27.50	Max.	12.37
		Min.	25.20	Min.	11.09

* Corrected to basis of cocoa butter having a Xylene No. of 0.19.

$$\frac{P-40}{100-40}=\frac{N-11.72}{26.18-11.72},$$

or

$$P=\frac{N-2.08}{0.241}, \text{ when } N \text{ is greater than } 11.72 \ (4).$$

Another series of mixtures of cocoa butter and butterfat was prepared, the samples shown as Cocoa Butter 1, and Butterfat 11 in Table 3 being used, and the xylene numbers were determined by the writer and another analyst. The results, with the percentages of butterfat calculated according to Equation (3), are shown in Table 4. It should be noted that the cocoa butter and butterfat used had xylene numbers differing widely from the average shown for each fat, so that the results in Table 4 show the behavior of the method under adverse rather than favorable conditions.

TABLE 4.

Results obtained by two analysts using the xylene number procedure.

BUTTERFAT	MODIFIED XYLENE NO.		BUTTERFAT CALCULATED	
	C. A. G.	J. I. P.	C. A. G.	J. I. P.
per cent			*per cent*	*per cent*
5	1.81 1.73	1.80 1.70	5.63 5.35	5.59 5.24
10	3.15 3.13	3.00 3.00	10.28 10.20	9.75 9.75
15	4.65 4.55	4.55 4.50	15.50 15.15	15.15 14.96
20	5.92 5.92	5.75 5.65	19.89 19.89	19.30 19.00
25	7.40 7.35	7.10 7.05	25.03 24.86	24.00 23.82

In applying this method to the examination of milk chocolate, the weight of sample that must be extracted to yield the required quantity of mixed fat can be calculated from the total fat determination. Having determined the percentage of milk fat in the extracted fat as described above, the percentage of milk fat in the entire sample is given by the formula:

$$M = \frac{F \times P}{100}, \text{ in which}$$

M = Percentage of milk fat in the sample;
P = Percentage of milk fat in the extracted fat; and
F = Percentage of total fat in the sample.

SUMMARY.

(1) A procedure is given for determining a modified xylene number on fats. This procedure determines the number of cubic centimeters of $0.1N$ alkali required to neutralize the volatile fatty acids obtained from 6.4 grams of the fat, which are neither precipitated as insoluble salts by magnesium sulfate nor extracted from an acidified aqueous solution by 20 per cent of its volume of xylene.

(2) Twelve samples of butterfat examined by this procedure gave values from 25.20 to 27.50, average 26.18, and six samples of cocoa butter gave values from 0.10 to 0.40, average 0.19.

(3) The percentage of butterfat in a mixture of butterfat and cocoa butter may be estimated by the equation:

$$\text{Percentage of butterfat} = \frac{\text{Xylene No.} - 0.19}{0.288},$$

if the xylene number is less than 11.72; or,

$$\text{Percentage of butterfat} = \frac{\text{Xylene No.} - 2.08}{0.241},$$

if the xylene number is greater than 11.72.

(4) Results obtained by this method are in close agreement in duplicate determinations and in those made by two independent analysts.

(5) The application of this method to the estimation of milk fat in milk chocolate is pointed out.

ACKNOWLEDGMENT.

A large number of the analyses reported in this paper were made by J. I. Palmore of this laboratory, and for his careful work the author is very grateful.

SECOND DAY.

TUESDAY—MORNING SESSION.

REPORT ON CHEMICAL REAGENTS.

By G. C. **Spencer** (Bureau of Chemistry, Washington, D. C.), *Referee.*

The Committee on Guaranteed Reagents of the American Chemical Society has continued its work of preparing specifications for reagent chemicals during the past year. These recommended specifications are adopted by the committee only after the proposed tests have been tried out in at least two of the laboratories that are represented and after thorough discussion by the committee.

The second publication of the committee has been published[1]. It describes the tests and limits of impurities for 23 reagents. Reference was made in the referee's report for 1925[2] to the first publication of the committee in that year, in which similar specifications were offered for 14 reagents.

Since October 1, 1925, the number of reagent chemicals rejected by the Bureau of Chemistry for unsatisfactory quality is 8 out of a total number of 238. Reasons for these rejections are briefly stated as follows:

Ether, U. S. P.—high residue.
Copper sulfate—excess of iron.
Ammonium chloride—furnished in paper carton.
Lead chromate—high water-soluble content.
Zinc—high arsenic content.
Lead acetate—excess of iron.
Sodium sulfite—foreign matter.
Cupric oxide—excess of chloride, sulfate, and calcium.

It is recommended that the observations on chemical reagents be continued[3].

REPORT ON EGGS AND EGG PRODUCTS.

By H. A. **Lepper** (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.), *Referee.*

The studies on eggs and egg products proposed in recommendations of the Committee on Recommendations of Referees at the last meeting were not completed owing to the late appointment of the referee and the change of assignment of J. C. Palmer, associate referee. No report is submitted by him this year. He recommends, however, a continuation of the studies to develop satisfactory methods for the following determinations:

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[1] *Ind. Eng. Chem.*, 1926, 8: 636 and 759.
[2] *This Journal*, 1926, 9: 347.
[3] For report of Sub-committee B and action of the association, see *This Journal*, 1927, 10: 66.

1. Water-soluble protein-nitrogen precipitable by 40 per cent alcohol.
2. Ash.
3. Unsaponifiable matter.

Collaborative study of successful methods already developed is also proposed.

H. I. Macomber of the New York Food and Drug Inspection Station, associate referee, whose report will follow, studied methods for the detection of decomposition of eggs. He recommends the adoption of a method for the determination of the acidity of the fat and further study of other methods.

DETERMINATION OF TOTAL SOLIDS.

Of the methods to be studied by the referee, opportunity was found to consider only those for determining total solids (moisture indirectly). A vacuum-oven method and an air-oven method for total solids in eggs have been adopted as official (first action), and tentative, respectively, with the recommendation for further study that will include the vacuum-oven method at 55°C., as given on p. 89 of Department of Agriculture Bulletin 846[1]. To obviate the uncertainties that might arise through changes taking place in egg samples furnished from a central source, the collaborators were asked to obtain their own samples of fresh whole eggs and yolks for determinations by the vacuum-oven method and routine air-oven method[2] and the 55°C. vacuum method for total solids. Fortunately, it was possible in almost every case to obtain two or more collaborators in the same laboratory to make the examinations desired. Their check results furnished the best means of judging the methods as to their reliability when placed in the hands of different workers. The results shown in Table 1 were obtained by analysts from the same laboratory and working on the same samples in each case.

COMMENTS BY COLLABORATORS.

R. L. Horst.—I prefer the electric oven at 112°–117°C., as it gives just as high moisture as the vacuum at the temperature of boiling water and it is more convenient to use. The vacuum at 55°C. gives results so much lower that they should be eliminated from the comparison.

N. E. Freeman.—Between the vacuum-oven method at 100°C. and the air-oven method at 112°C. there is very little difference. If anything, the vacuum-oven method gives very slightly higher results, but the difference is so slight as to be negligible and not worth the extra trouble. The vacuum oven at 55°C. gives results that are low by amounts varying from 0.3 to 0.8 per cent, and so they would not appear to be reliable. For these reasons I am in favor of using the air-oven method at 112°C.

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[1] *This Journal*, 1926, 9: 83.
[2] *Ibid.*, 56.

TABLE 1.

Total solids in eggs.

COLLABORATOR	FRESH WHOLE EGGS			FRESH EGG YOLKS		
	Vacuum 98°–100°C.	Air-Oven 112°–117°C.	Vacuum 55°C.	Vacuum 98°–100°C	Air-Oven 112°–117°C	Vacuum 55°C.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H. W. Haynes, U. S. Food and Drug Inspection Station, Boston, Mass.	26.53* 26.51	26.40 26.51	26.95 26.89	50.62* 50.50	50.42 50.34	50.82 50.93
C. H. Hickey, U. S. Food and Drug Inspection Station, Boston Mass.	26.65* 26 59	26.53 26.54	27.23 27.13	50.62* 50.61	50 42 50.47	50.98 51.03
E. L. P. Treuthardt, U. S. Food and Drug Inspection Station, Boston, Mass.	26.40* 26.56	26.21 26.30	26.96 27.02	50.53* 50.53	50.49 50.51	51.00 50.93
H. R. Smith, U. S. Food and Drug Inspection Station, Baltimore, Md.	26.93 26.79	26.88 26.90	27.32 27.36	45.67 45.61	45.54 45.56	46.14 46.18
T. F. Pappe, U. S. Food and Drug Inspection Station, Baltimore, Md.	26.83 26.76	26.88 26.87	27.43 27.49	45.59 45.66	45.59 45.51	46.11 46.11
R. L. Horst, U. S. Food and Drug Inspection Station, New Orleans, La.	25.74 25.74 25.68	25.70 25.68 25.65	26.13 26.03 26.03	50.53 50.42	50.59 50.57	51.33 51.25 ....
N. E. Freeman, U. S. Food and Drug Inspection Station, New Orleans, La.	25.74 25.69	25 77 25.73 25.70	26.13 26.09 26.04	50.50 50.50	50.55 50.54 50.51	51.37 51.29 .
I. C. Mitchell, U. S. Food and Drug Inspection Station, St. Louis, Mo.	26.07 26.22	26.21 26.24	26.56 26.57	47 72 47.75	47.77 47.80	48.59 48.49
S. Alfend, U. S. Food and Drug Inspection Station, St. Louis, Mo.	26.27 26.34	26.40 26.41	26.45 26.52	47.86 47.95	47.91 48.04	48.13 48.13
D. B. Scott, U. S. Food and Drug Inspection Station, New York, N. Y.‡	27.89* 27.96	27.62 27.55	27.65 27.61	49.17* 49.01	48.55 48.48	48.74 48.86
H. I. Macomber, U. S. Food and Drug Inspection Station, New York, N. Y.‡	28.00* 28.01	27.04 27.39	27.06 27.54	48.75* 48.81	48.31 48.24	48.37 48.32
C. E. Goodrich, Food Control Laboratory, Bureau of Chemistry, Washington, D. C.	27.77 27.79	27.99 27.84	28.37 28.32	49.02 48.91	49.22 49.19	49.68 49.68
J. I. Palmore, Food Control Laboratory, Bureau of Chemistry, Washington, D. C.	27.65 27.66	27.72 27.88	28.20 28.31	49.03 49.01	49.25 49.79	49.80 49.71
T. O. Kellems, U. S. Food and Drug Inspection Station, San Francisco, Calif.	26.84 26.82	26.79 26.69	27.25 27.20	47.67 47.64	47.62 47.61	48.96 48.69
L. H. Chernoff, U. S. Food and Drug Inspection Station, Denver, Colo.	27.87†	27.72	28.06	48.23†	48.24	48.71
L. Feldstein, U. S. Food and Drug Inspection Station, Denver, Colo.	27.80†	27.64	28.15	48.44†	48.24	49.01

* Vacuum 27 inches.
† Temperature 94°C.
‡ Results by these collaborators on frozen eggs and yolks

L. C. Mitchell.—Due to hot weather it was impossible to maintain a temperature of 55°C. It was 55°C. at the beginning of the period of drying and 66°C. at the end. Besides results on whole eggs and yolks, results were obtained on fresh whites as follows: vacuum 98°-100°C.—11.57 and 11.57; air oven 112°-117°C.—11.55 and 11.53; and vacuum 55°C.—11.77 and 11.74 per cent.

D. B. Scott and H. I. Macomber.—The eggs used were samples of imported frozen eggs.

L. D. Elliott.—From the results of L. H. Chernoff and L. Feldstein it appears true that the vacuo—55°C.—gives consistently higher results than either of the other two methods, and these other two methods check relatively closely with each other. There is a wider difference between the 55°C. method and the other two in the case of yolk than there is in the case of whole eggs, which might suggest that the difference is due to a slight volatilization of fat in the case of the 94° and 112°C. methods, rather than to a retention of moisture in the 55°C. method.

T. O. Kellems.—Drying in the electric oven at 115°C, and in the vacuum oven at 98°-100°C. was complete at the end of the first drying (3½ hours). A second drying of ½ hour showed no further loss in weight. This was true for both whole eggs and egg yolk. The results by the vacuum oven at 55°C. were obtained by drying for a total of 6½ hours. The first drying was for 2½ hours, and the other periods were from ½ to 1 hour. The checks obtained, the time required, and the ease of manipulation are all in favor of the use of covered dishes and either the vacuum oven at the temperature of boiling water or the electric oven at 112°-117°C. The egg loses moisture at the rate of about 1 mg. per minute from a 5 gram sample while weighing in an open dish, and will gain at about the same rate after being dried.

DISCUSSION.

The minimum and maximum variations in the checks obtained by individual analysts for the three methods on whole eggs and yolks are given in Table 2. This table also includes the calculated average of the total variations. Similar calculations made on the differences in the results reported by the analysts working on the same sample are given in Table 3. These values do not include the results of Scott and Macomber on frozen eggs.

TABLE 2.

Variations in checks of individual analysts.

OVEN USED	WHOLE EGGS			YOLKS		
	Minimum	Maximum	Average	Minimum	Maximum	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Vacuum at 98°C.	0.00	0.16	0.06	0.00	0.12	0.06
Air-oven	0.01	0.25	0.07	0.01	0.13*	0.05*
Vacuum at 55°C.	0.00	0.11	0.06	0.00	0.16	0.06

* The one discordant difference of 0.54 per cent is not included.

There appears to be no choice in the methods from the point of view of the ability of the analyst to check himself. The variations among

TABLE 3.

Variations in results by different analysts on the same sample.

	WHOLE EGGS			YOLKS		
	Minimum	Maximum	Average	Minimum	Maximum	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Vacuum at 98°C....	0.00	0.15	0.08	0.01	0.21	0.08
Air-oven..	0.01	0.27	0.12	0.00	0.18	0.07
Vacuum at 55°C..	0.02	0.26	0.12	0.03	0.41	0.18

analysts are somewhat closer, especially on yolks, by the vacuum-oven at 98°C. and air-oven methods than by the vacuum oven at 55°C. While the results on frozen eggs by Scott and Macomber do not exactly parallel the general findings on fresh eggs, they do not deviate sufficiently to permit the drawing of different conclusions with respect to the application of the methods to frozen products. Each analyst found the vacuum oven at 55°C. to give higher results than either the vacuum oven at 98°C. or the air oven on both whole eggs and yolks. The vacuum oven at 98°C. sometimes gave higher and sometimes lower results than the air-oven method. These findings confirm those of the previous referee[1]. The minimum and maximum differences in the results by the various methods, as well as the averages of these differences, are given in Table 4. In comparing the air-oven method and the vacuum-oven method at 98°C., the minimum values given represent the greatest variation found when the result by the vacuum oven was greater than by the air oven, while the maximum represents the greatest variation when the reverse condition existed. In calculating the averages in this case, the differences were all regarded as being of the same nature.

TABLE 4.

Comparison of results by the different methods.

SAMPLE	VACUUM AT 55°C. AND VACUUM AT 98°C			VACUUM AT 55°C. AND AIR OVEN			AIR OVEN AND VACUUM AT 98°C		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Whole eggs	0.18	0.70	0.44	0.33	0.75	0.46	0.22	0.14	0.09
Yolks..... ..	0.23	1.18	0.58	0.22	1.22	0.59	0.28	0.52	0.13

The higher results given by the vacuum oven at 55°C. over the two other methods have been the subject of comment by the collaborators.

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[1] *This Journal*, 1926, 9: 354.

It is pointed out by some that all the moisture is not driven out at 55°C. and by others that the fat of the yolk is volatilized by 98° or 112°C., giving a value too low for total solids. As the merits of these claims have not been verified by experimental work, at present no choice of the methods is possible on the question of efficiency of moisture removal without loss of weight due to decomposition of egg material. Reasons for the adoption of the vacuum-oven at 98°C. and the air-oven methods have been advanced by Hertwig, referee in 1924[1] and 1925[2]. Remarks by Redfield[3] at last year's convention pointed out certain disadvantages in adopting these methods. While the collaborative results warrant the adoption of the present official and tentative methods, the work this year has not furnished information on the remaining doubtful points, especially on the efficiency of moisture removal, to make final action possible.

The method for acidity of the fat recommended for tentative adoption by Associate Referee Macomber is essentially the method recommended by Redfield in Bulletin 846. Collaborative data are given to support the recommendation. This method includes a determination of total solids in vacuum at 55°C., a procedure for determining total solids differing from the two other methods given previously in this report and now in effect. While an effort should be made to keep the methods of analysis as concordant as possible, it should be remembered that methods of examination are of little value in control activities unless authentic data are available by which interpretations can be made. In the case of the method recommended for the determination of acidity of fat, such data have been accumulated in Bulletin 846. No similar data are at hand, however, for acidity of the fat as determined on residues dried at 98°C. or 112°C. For this reason the recommendation appears to be worthy of adoption for the present, but it should be indicated in the methods of analysis that this method serves a single purpose. At the same time, the generally applicable methods for total solids already in effect can be retained until such time as study may show them to be less accurate than the 55°C. method, which is included in the acidity of the fat procedure.

RECOMMENDATIONS[4].

It is recommended—

(1) That studies be made by the associate referees for purposes of perfecting methods for the determination of—

(a) water-soluble protein-nitrogen precipitable by 40 per cent alcohol,

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[1] *This Journal*, 1925, 8: 594.
[2] *Ibid.*, 1926, 9: 348.
[3] *Ibid.*, 352.
[4] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 73.

(b) ash, and

(c) unsaponifiable matter,

and to include, if possible, collaborative studies of such methods and of the present methods for the determination of fat (acid hydrolysis) and of lipoids and lipoid phosphoric acid.

(2) That further study be given to the vacuum-oven at 98°C., the air-oven, and the vacuum-oven at 55°C. methods for the determination of total solids to ascertain the efficiency of each with respect to actual moisture removal.

(3) That the method for the determination of acidity of the fat, given in the report of H. I. Macomber, be adopted as a tentative method, that it be included in *Methods of Analysis* in the chapter on "Eggs and Egg Products" under the subheading, "Methods for the Detection of Decomposition", and that study by the associate referee be continued on other methods for detecting decomposition to be included in this subdivision.

No report on water-soluble protein-nitrogen precipitable by 40 per cent alcohol, unsaponifiable matter, and ash was given by the associate referee.

REPORT ON THE DETECTION OF DECOMPOSITION IN EGGS.

By H. I. Macomber (Food and Drug Inspection Station, New York, N. Y.), *Associate Referee.*

Several methods for the detection of decomposition in liquid eggs were studied by H. W. Redfield and his associates and reported in U. S. Department of Agriculture Bulletin 846. Three of these methods that have been used by the Bureau of Chemistry for several years in the examination of frozen egg products are those for the determination of ammonia nitrogen, of reducing substances as dextrose, and of the acidity of the fat. These determinations, together with a bacteriological examination, have been found to give a reliable indication as to spoilage or the use of decomposed eggs in a frozen egg product.

However, in the case of dried eggs the detection of decomposition has been somewhat more difficult. The ammonia nitrogen is driven off in varying degrees by the heat used in drying, so that the results obtained are high or low, depending upon drying conditions rather than upon the condition of the eggs. The results obtained in the determination of reducing substances are also erratic and are not to be depended upon as indications of decomposition. Unpublished results of an investigation conducted by the New York Station of the Bureau of Chemistry, in which authentic dried egg samples were used, show that drying has little, if any, effect on the acidity of the fat and that this determination can be depended upon to detect decomposition that was present in the eggs before drying or that occurred in the dried product during storage. This method is, of course, not applicable to egg white either before or after drying.

Another method that has been developed for the detection of spoilage in dried and whole egg is the determination of acid-soluble phosphoric acid. This method was adapted to eggs from the Chapin-Powick method by Louis Pine and is described in his paper published previously in this Journal[1]. His method, originally used for liquid eggs, was modified slightly for dried eggs and found to be an excellent index of the extent of decomposition.

These two methods, the acidity of the fat and the acid-soluble phosphoric acid, were sent out for collaborative study by the associate referee in 1924[2], and the results were reported at the meeting in that year. The results were not very satisfactory, and it was recommended that the methods be given further study. Both methods have been modified and simplified, and this year collaborative data have been secured on the determination of the acidity of the fat. The method for the determination of acid-soluble phosphoric acid was not ready in time to send out to collaborators. The method for the determination of the acidity of the fat is essentially that given in Bulletin 846, page 90, and is as follows:

DRIED EGGS.

REAGENTS.

(a) *Anhydrous ethyl ether.*—Prepare in the usual way from ordinary ethyl ether.

(b) *Benzol.*—Use the best quality benzol available. If it is not neutral, titrate 50 cc. with the 0.05 N sodium ethylate and correct results accordingly.

(c) *0.05 N sodium ethylate.*—Dissolve a piece of metallic sodium, approximately 1 cc. in volume, in 800 cc. of absolute alcohol. Titrate 10 cc. of 0.10 N hydrochloric acid with this solution and add the calculated amount of absolute alcohol to make the solution 0.05 N. Ascertain the normality factor by titrating against 0.10 N hydrochloric acid on the day the solution is used.

DETERMINATION.

Weigh 2 grams of powdered dried egg into a tared aluminum dish of about 2½ inches diameter, and dry in a vacuum of not less than 25 inches at 55°C., until there is no further loss in weight. Make the first weighing at the end of 2 hours and further weighings at half-hour intervals. Weigh to three decimal places.

Extract the dried residue with absolute ether, preferably in a Knorr apparatus. Carefully transfer the egg powder to a 12.5 cm. hardened filter paper, fold the filter paper once, place on a 15 cm. qualitative filter paper, and roll the filter papers and contents into a cylinder which will fit snugly into the extraction tube, folding in one side of the large filter paper to prevent loss of material. An asbestos plug is not needed in the extraction tube. If the extractor is working rapidly, three hours is sufficient to insure proper extraction. Evaporate the ether from the extraction flask, and dry the extract for 1 hour at 55°C. in a vacuum of not less than 25 inches. Weigh to three decimal places.

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[1] *This Journal*, 1924, 8: 57.

[2] *Ibid.*, 1925, 8: 604.

Dissolve the ether extract obtained as above in 50 cc. of the benzol and add three or four drops of phenolphthalein indicator. Titrate with the 0.05 *N* sodium ethylate, expressing the results as cc. of 0.05 *N* sodium ethylate per gram of ether extract. The end point is reached when the yellow of the ether extract changes to orange.

LIQUID EGGS.

The method for determining acidity of the fat in liquid eggs is the same as that used for dried eggs with the following exceptions:

Weigh a sample of approximately 5 grams accurately to three decimal places into a tared lead dish. Make the first weighing after drying for about 5 hours and at intervals of 1 hour thereafter.

In preparing the dried residue for extraction with ether, place the lead dish upon a 12.5 cm. hardened filter paper, cut the sides of the dish through at four equidistant points, and flatten down. Place another similar filter paper on top of the lead dish and roll the papers and dish into a cylinder that will fit snugly into the extraction tube. In making the cylinder one side must be tucked in to prevent any of the egg dropping into the extraction flask.

Authentic samples of drum-dried yolk and whole egg which had been stored at room temperature for nearly three years were sent to five collaborators. Because of the difficulty involved in shipping liquid eggs, collaborators were asked to determine the ether extract and acidity of the fat from the dried residue obtained in determining moisture at 55°C., as requested by H. A. Lepper, the Referee for Eggs and Egg Products. The results obtained were as follows:

Collaborative work on acidity of fat.

(Results expressed as cc. of 0.05 *N* sodium ethylate per gram of fat.)

COLLABORATOR	DRIED EGGS		LIQUID EGGS	
	Whole egg	Yolk	Whole egg	Yolk
H. R. Smith Food and Drug Inspection Station Baltimore, Md.	11.1	5.5	1.61	1.73
L. A. Salinger Food and Drug Inspection Station Savannah, Ga.	11.4	5.1		
J. I. Palmore Bureau of Chemistry Washington, D. C.	10.80	5.05	2.40	2.10
C. E. Goodrich Bureau of Chemistry Washington, D. C.	10.93	5.03	2.33	2.16
D. B. Scott Food and Drug Inspection Station New York, N. Y.		. .	2.72	3.12
H. I. Macomber	11.46	5.14	1.84	1.98

In the case of the dried eggs the agreement among the various collaborators is quite close, especially the results on the yolk. The results obtained on the liquid eggs cannot be compared in the same way because the samples were not the same, as has been explained. H. R. Smith's results were not checked by any other analyst, but they are normal for good fresh eggs. It will be noted that the results obtained by J. I. Palmore and C. E. Goodrich on the same sample agree very closely. The liquid eggs analyzed by Scott and the writer were the same sample of imported frozen eggs. The variation in results of about 1 cc. is accounted for by the fact that her analysis was begun one day later than the writer's, during which time decomposition took place.

None of the collaborators offered any comments or criticisms of the method.

RECOMMENDATIONS[1].

It is recommended—

(1) That the method for the determination of the acidity of the fat, as described in this report, be adopted as a tentative method[2].

(2) That the method for the determination of acid-soluble phosphoric acid, as described in this report, be given further study with the object of securing collaborative data.

(3) That a study be made of the other methods for the detection of decomposition in eggs mentioned in this report, namely, ammonia nitrogen and reducing substances as dextrose, with the object of securing collaborative data.

The report of the Associate Referee on Total Solids in Eggs, H. A. Lepper, is included in his report as Referee on Eggs and Egg Products, p. 406.

REPORT ON FOOD PRESERVATIVES.

By WYATT W. RANDALL (State Department of Health, Baltimore, Md.), *Referee.*

In his report to this association at the meeting of 1925 the referee recommended a further study of the sublimation method for the separation, purification, and determination of benzoic acid, salicylic acid, and saccharin, when they or their derivatives are used as preservatives in food products. The work which it was found possible to do was confined to the determination of benzoic acid in ketchup.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 73.

[2] *This Journal*, **50**.

In the endeavor to improve upon the method of filtration suggested by the official method for the ketchup: salt-solution mixture, various experiments were conducted. Squeezing this mixture within a muslin bag did not appeal to the referee as an ideal procedure. When stout close-woven material was employed, much of the liquid remained with the solid matter within the bag, or else was absorbed in the muslin itself. If, on the other hand, a fabric less closely woven was used, so much of the fine, fibrous tomato pulp passed through that the process appeared of little value. Again, evaporation from the wet muslin surface may be a source of considerable inaccuracy. Filtration through paper in the usual way is extremely slow, and much of the fluid remains mixed with solid material in the filter even after many hours' draining. Accordingly, resort was had to Büchner funnels and the use of strong suction. Two filter papers were used in each funnel, and a ring of proper size, cut from rubber cloth, was placed upon them: the ring holds down the edges of the papers and so prevents the inclusion of any of the pulp under them when the fluid mass is poured in, while it leaves practically all the perforated surface of the plate available for its work. At first the liquid passes through rapidly, but as a mat of solid material collects on the paper, the process slows up, as would be expected. Of course it is desirable to use a large funnel so as not to delay the process. A perfectly clear filtrate is readily secured, and this, in the opinion of the referee, is very desirable as tending to lessen the formation of annoying emulsions when extraction with chloroform is undertaken.

An obvious advantage which this method of filtration possesses is that practically *all* the fluid is separated from the solid matter; the latter can then be washed with saturated salt solution and the filtrate ultimately made up to a definite volume, an aliquot of which then represents a known fraction of the original sample weighed. While in the official method only a small, perhaps negligible, error is introduced through the employment of a given volume of the filtrate as if it were the same (in benzoic acid content) as an equal volume of the unfiltered mass, nevertheless it would seem advisable to make the volume up to a fixed point *after* filtration rather than *before*.

Another matter calling for study, in view of the caution advised by the framers of the official method, is the amount of vigor to be employed in "shaking out" with chloroform. What does "careful extraction" or "cautious shaking" imply?

PRELIMINARY WORK.

Three ketchups were prepared: In A there was dissolved 0.100 per cent, in B 0.060 per cent, of pure benzoic acid; none was added to C. Of each of these samples 100 grams was weighed off, rendered alkaline, treated with salt and with saturated salt solution until a volume of

TABLE 1.

Preliminary determinations.

Sample	A			B			C		
Benzoic acid added (per cent)	0.100			0.060			None		
Weight of crude chloroform extract (gram)	0.0429	0.0477	0.0450	lost	0.0254	0.0335	0.0065	0.0193	0.0321
Loss in weight of dish and contents during sublimation (gram)	0.0316	0.0308	0.0333	. ..	0.0155	0.0227	0.0066	0.0068	0.0177(?)
Hence, percentage of benzoic acid	0.105	0.103	0.111	.. ...	0.052	0.076	0.022	0.023	?
Weight of sublimate recovered (gram)	0.0350	0.0339	lost	0.0236	0.0199	0.0218	0.0076	0.0064	0.0029
Hence, percentage of benzoic acid	0.117	0.113	.. ..	0.079	0.066	0.073	0.025	0.021	0.010
Weight of residue in dish after sublimation (gram)	0.0093	0.0169	0.0117	lost	0.0099	0.0108	0.0125	0.0125	0.0144
Weight of benzoic acid, by titration (gram)	0.0266	0.0257	... .	0.0155	0.0146	0.0152	0.0015	0.0006	0.0008
Hence, percentage of benzoic acid	0.089	0.086	..	0.052	0.049	0.051	0.005	0.002	0.0027

about 450 cc. was obtained, and then filtered; the filter was washed with the salt solution, and the total filtered liquid in each case was brought to a volume of 500 cc. Three aliquots, of 150 cc. each, were used in determining benzoic acid in each of the three prepared solutions. Violent agitation during the extraction process was avoided, but five or six extractions were resorted to, instead of four. Table 1 shows the results. The first two columns in each case show the referee's determinations, the third those made by W. H. Schulze, of the State of Maryland Department of Health.

Examination of the figures in Table 1 leads to certain conclusions:

1. A notable quantity of material, which was not benzoic acid, was volatilized from the dried chloroform extract.
2. A considerable amount of nonvolatile material was contained in the chloroform extract.
3. Part of the benzoic acid may have been volatilized past the condensing bulb and so lost; in other words, sublimation may have been conducted too rapidly or at too high a temperature.
4. The benzoic acid may not have been completely extracted from the aqueous solution.
5. Owing to the presence of spice oils and lubricating grease in the chloroform extract, part of the benzoic acid therein may not have been volatilized and hence failed to show up on titration of the sublimate.

COLLABORATIVE WORK.

To test the proposed process of filtration and the sublimation method for the purification of the chloroform extract, and to obtain the criticism of others, three samples of ketchup were prepared, and similar amounts of each of these were put in the hands of the following collaborators: W. C. Johnson of the Minnesota State Laboratory, V. B. Bonney of the U. S. Bureau of Chemistry, W. H. Schulze, and the referee. The following instructions were issued with the samples:

COOPERATIVE WORK ON THE DETERMINATION OF SODIUM BENZOATE.

The referee desires to try out by cooperative work the following method for the determination of benzoic acid (or of sodium benzoate) added to tomato ketchup. The official method given on pages 128-129 of *Methods of Analysis* (X, **10** (a), **11**), is to be used with the following modifications:

(1) In place of **10** (a), as printed, use the following:

10 (a) *Ketchup.*—To 150 grams of the ketchup, in a proper-sized flask, add 15 grams of pulverized sodium chloride and about 300 cc. of clear saturated sodium chloride solution. Mix thoroughly, render alkaline to litmus paper by means of 10 per cent sodium hydroxide solution, shake violently from time to time during at least 2 hours, filter[1] with the aid of a pump, transfer the clear filtrate to a 500 cc. volumetric flask, wash the material on the filter with small quantities of saturated salt solution, add the washings to the main filtrate until the flask has been filled to the mark, and mix thoroughly.

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[1] A satisfactory device for this purpose is a 4 inch Büchner funnel provided with a double filter paper of proper size, upon which is placed a ring cut from rubber cloth of about 1 mm thickness The width of the ring should be about 1 cm. and its outside diameter a little less than that of the funnel diaphragm. It serves to hold down the edges of the filter papers while the liquid is being poured into the funnel If the first portion of the filtrate collected is cloudy, it should be passed through the filter a second time.

(2) In section **11**, follow the directions of the text of the official method through the first paragraph and the first two sentences of the third paragraph and then continue as directed in the following proposed new paragraph to be inserted:

"The residue (mentioned in the last sentence of the third paragraph) may be transferred, instead, to the dish designed for the Hortvet sublimator and, after evaporation of all the solvent, subjected to sublimation under a pressure of not more than 5–10 mm. of mercury, the temperature of the sublimator bath being allowed to rise slowly to 110°–115°C. When the operation has been conducted long enough to secure the removal of all volatile material from the dish and after the apparatus has cooled and has been disconnected, the sublimate is washed, by means of chloroform, from the bulb into a glass dish or beaker, from which the solvent is allowed to evaporate spontaneously."

(3) Next, follow the last paragraph of section **11** throughout with the following exception: After the words "with 0.05 N sodium hydroxide", insert the following clause: "(preferably standardized against an alcoholic solution of pure benzoic acid)".

Of the ketchup samples distributed, two contain added benzoic acid; one contains no added benzoic acid and is to serve as a blank.

It is desired that the collaborating chemists make the following determinations with each of the three samples:

(1) Find the weight of the residue left on evaporation of the chloroform used in the extraction of benzoic acid from the ketchup.

(2) Find the loss in weight of the sublimator dish and its contents as a result of the sublimation process.

(3) Find the weight of the sublimate washed from the sublimator, after the evaporation of the solvent used.

(4) Find the weight of the benzoic acid in this residue, through titration by means of standard alkali solution.

Tables 2, 3, and 4 contain the results obtained by Johnson, Bonney, and Schulze; the referee's results were not complete at the time this report draft was written.

Johnson comments upon the method prescribed as follows:

> The results included in the tabulation were obtained by following closely the instructions given by the referee. Great difficulty was experienced with the method of filtering on account of the tendency of the tomato pulp to form a densely packed layer over the surface of the filter paper. Using a double filter in the manner directed, at least 12 hours' time was required in order to complete the filtration and washing. Owing to repeated attempts and failures and the necessity of using additional water incidental to changing filter paper, an excessive amount of filtrate was obtained in the case of Ketchups A and C. Evaporation was resorted to in order to adjust the volume to the required 500 cc. The result was a considerable darkening of the solution. As a rule, good separations of the chloroform layer were secured after proper shaking and manipulation of the separatory funnel. In connection with Ketchup B, Table 3, an attempt to improve the separation by running the funnel in the centrifuge resulted in the loss of one of the portions. [This portion is the second under "Official" (Johnson).]
>
> There is needed an improvement in the preparation of the sample for filtration. Some investigations are under way with the view to effecting this improvement, and if satisfactory results are obtained a description of the improvement will be submitted at a later date.

TABLE 2.

Collaborative work.

(Sample A —Benzoic acid added. 0.155 per cent)

Method . . .	Official		Sublimation							Official
Collaborator	Johnson		Johnson		Bonney			Schulze		Schulze
Volume of solution used (cc.)	100	100	100	100	100	100	100	150	150	150
Weight of crude chloroform extract (gram)			0.0570	0.0542	0.0499	0.0465	0.0480	0.0796	0.0833	
Loss in weight of dish and contents during sublimation (gram) .			0.0472	0.0462	0.0459	0.0447	lost	0.0612	0.0635	
Hence, percentage of benzoic acid .			0.157	0.154	0.153	0.149	. .	0.136	0.141	
Weight of sublimate (gram) . .			0.0466	0.0454	0.0436	0.0446	. .	0.0597	0.0612	
Hence, percentage of benzoic acid			0.155	0.151	0.145	0.149	.	0.133	0.136	
Weight of residue in dish after sublimation (gram)			0.0098*	0.0080*	0.0040	0.0018		0.0184	0.0198	
Volatile matter other than sublimate (gram)			0.0006	0 0008	0.0023	0.0001		0.0015	0.0023	
Weight of benzoic acid by titration (gram)	0.0467	0 0478			0.0405	0.0405		0.0571	0.0584	0 0612
Hence, percentage of benzoic acid	0.156	0.159			0.135	0.135	.	0.127	0.130	0.136

* Qualitative tests applied to these residues failed to show presence of benzoic acid

TABLE 3.

Collaborative work.

(Sample B.—Benzoic acid added: 0.076 per cent.)

Method	Official		Sublimation							Official
Collaborator	Johnson		Johnson		Bonney			Schulze		Schulze
Volume of solution used (cc.)	100	100	100	100	100	100	100	150	150	150
Weight of crude chloroform extract (gram)			0.0261	0.0299	0.0280	0.0274	0.0277	0.0568	0.0620	
Loss in weight of dish and contents during sublimation (gram)			0.0228	0.0241	0.0214	0.0218	0.0216	0.0302	0.0310	
Hence, percentage of benzoic acid			0.076	0.080	0.071	0.073	0.072	0.067	0.069	
Weight of sublimate (gram)			0.0224	0.0229	0.0212	0.0214	0.0215	0.0304	0.0270†	
Hence, percentage of benzoic acid			0.075	0.076	0.071	0.071	0.072	0.068	0.060	
Weight of residue in dish after sublimation (gram)			0.0033*	0.0058*	0.0068	0.0060	0.0062	0.0266	0.0310	
Volatile matter other than sublimate (gram)			0.0004	0.0012						
Weight of benzoic acid, by titration (gram)	0.0240	lost			0.0209	0.0209	0.0209	0.0274	0.0240†	0.0316
Hence, percentage of benzoic acid	0.080	...			0.070	0.070	0.070	0.061	0.053	0.070

* Qualitative tests applied to these residues failed to show presence of benzoic acid.
† Spattering caused a loss of material.

TABLE 4.

Collaborative work.

(Sample C.—No benzoic acid added.)

Method	Official		Sublimation							Official
Collaborator	Johnson		Johnson		Bonney			Schulze		Schulze
Volume of solution used (cc.)	100	100	100	100	100	100	100	150	150	150
Weight of crude chloroform extract (gram)			0.0088	0.0100	0.0049	0.0047	0.0049	0.0165	0.0150	
Loss in weight of dish and contents during sublimation (gram)			0.0040	0.0052	0.0010	0.0007	0.0009	0.0029	0.0029	
Hence, percentage of benzoic acid			0.013	0.017	0.003	0.002	0.003	0.006	0.006	
Weight of sublimate (gram)			0.0024*	0.0022*	0.0013	0.0008	0.0008	0.0004	0.0008	
Hence, percentage of benzoic acid			0.008	0.007	0.004	0.003	0.003	0.001	0.002	
Weight of residue in dish after sublimation (gram)			0.0048†	0.0048†	0.0039	0.0040	0.0040	0.0136	0.0121	
Volatile matter other than sublimate (gram)			0.0016	0.0030						
Weight of benzoic acid, by titration (gram)	0.0024	0.0024	0.0010	0.0010	None	None	None	0.0006	0.0012	0.0024
Hence, percentage of benzoic acid	0.008	0.008	0.003	0.003	None	None	None	0.001	0.002	0.005

* This material, on titration, proved to be less than half acid (calculated as benzoic).

† Qualitative tests applied to these residues failed to show presence of benzoic acid.

Much inconvenience was experienced owing to the smallness of the samples submitted. It was impossible to arrange for duplicate determinations other than those shown in the tabulation. After proper drawing out of portions I, II, III, IV, there usually remained an insufficient volume for an additional determination. It seems likely that the instructions given in the official method for making up the sample to 500 cc., then continuing with the filtration, may be the better procedure. This mode of operation eliminates considerable delay owing to the fact that the analyst, after taking his first aliquot portion, may proceed with the extraction while the filtration continues.

The tabulated results reveal the following:

(1) The weighed residue in the dish after evaporation of the chloroform includes, in addition to benzoic acid, a substantial amount of other acid-reacting material.

(2) A notable amount of material disappears during the sublimation. This material doubtless includes volatile acid and/or volatile oil derived from the spices used in the preparation of the ketchup.

(3) Titration of the residue according to the official method yields too high a result owing to the presence of impurities indicated under (1) and (2).

(4) The results obtained on Ketchup C reveal the presence of volatile matter which tends to contaminate the sublimate of benzoic acid. The figures in the last two columns (3 and 4), obtained by weighing, are, however, misleading. The material represented by 0.0024 and 0.0022, respectively, by weight, when dissolved in water-alcohol mixture was only slightly acid and, when titrated, amounted in each case to 0.0010. It appears, therefore, that the sublimate of benzoic acid should not be weighed, but should be subjected to titration after washing from the bulb with strong alcohol and diluting with water as in the official procedure. The error will thereby be greatly reduced.

(5) The conclusion appears to be that the sublimation procedure, introduced at the point indicated in the instructions of the referee, results in a purification of the extracted benzoic acid whereby the interfering acid-reacting material is practically eliminated.

The instructions given by the referee require a gradual heating of the sublimator bath to a temperature of 110°–115°C. This heating is undoubtedly excessive and tends to increase the liberation of impurities which are condensed with the sublimate. Actual observations made on pure benzoic acid indicate that the temperature need not exceed 75°–80°C. By using a specially constructed thermometer fitted inside the sublimator, it has been observed that benzoic acid begins to sublime at 35°C. under a 0.05 mm. vacuum. The sublimation would be completed by holding the temperature at 35°C. for a sufficient time. However, by raising the temperature gradually to 75°C., the sublimation is completed much sooner. In the meantime it is observed that the temperature recorded by the control thermometer (outside) is uniformly higher than that recorded inside the sublimator. When toward the conclusion of the operation the flame is adjusted to maintain a final temperature of 75°C. the two thermometer readings tend to approach each other. The major portion of the sublimate is collected inside of 10–15 minutes, and the sublimation operation is usually completed in approximately one-half hour.

The referee is of the opinion that Johnson's results are unusually accurate. The fact that his determinations are in practically every case closer to the actual amount of benzoic acid present than are those of the other collaborators—and are always higher—indicates that he succeeded in extracting the acid completely where the others failed so to do. Inquiry brought out the fact that interpretations of the directions for the extraction process may vary in a marked degree. Johnson, it was learned, "shook vigorously for about a minute"; Schulze and the referee took great pains to mix the liquids thoroughly, yet with as little violent agitation as possible. The results would appear to show that the former process is not only more efficient—as would be expected—but that it can be applied without danger of the formation of unmanageable emulsion.

CONCLUSIONS.

The referee is of the opinion that the results given show that:

1. Benzoic acid is not completely extracted from the ketchup: salt-solution mixture by means of chloroform, unless vigorous shaking is employed.

2. From this mixture chloroform extracts acid-reacting substances other than benzoic acid.

3. The official method, therefore, may indicate the presence of small amounts of benzoic acid when, as a matter of fact, it is absent.

4. The sublimation method goes far to separate any extracted benzoic acid from other acid-reacting substances which may be dissolved by the chloroform used, and hence is an excellent check upon the official method.

5. The smallest possible amount of lubricant should be used for separator stopcocks and for sealing together the two parts of the sublimator.

6. The determination of the benzoic acid present in a ketchup: (a) from the loss suffered on sublimation by the chloroform extract, or (b) from the weight of the material sublimed, is as accurate as that by the official method.

7. The determination of such benzoic acid through the titration of the acid material separated by sublimation from the dried chloroform extract is more accurate than is either of the determinations mentioned in 6.

8. The "proposed method" sent out to the several collaborators for trial calls for modification:

   (a) In its directions for conducting the process of extraction by means of chloroform;
   (b) In its statement of the temperature and pressure to be used in the process of sublimation; and
   (c) Possibly in other details.

9. A definite analytical procedure involving the sublimation process should not be recommended at this time, but another effort should be made by collaborative work to settle certain matters of detail, in the hope that a tested procedure may be recommended for adoption a year hence.

RECOMMENDATIONS[1].

The referee recommends—

(1) That further collaborative study be devoted to the process of removing benzoic acid from ketchup with the aid of chloroform.

(2) That further collaborative study be devoted to the process of subliming benzoic acid, in order that optimal temperature and pressure conditions may be established.

(3) That the official method for the determination of benzoic acid in products other than ketchup be compared with methods involving the sublimation process.

(4) That, as soon as possible, the sublimation process be applied to the determination of salicylic acid and to that of saccharin (in food samples into which these substances have been introduced) and that suitable procedures for their determination be devised.

REPORT ON COLORING MATTERS IN FOODS.

By C. F. JABLONSKI (U. S. Food and Drug Inspection Station, New York, N. Y.), *Referee.*

Owing to the unusual pressure of regulatory work the recommendations of the committee were only partially carried out.

Recommendation 1, "that collaborative work be undertaken on the separation of light green S. F. yellowish from guinea green B and yellow O B from A B" was not undertaken, but D. B. Scott of the New York Laboratory submitted the results of a number of experiments on the separations of yellow A B and yellow O B. The results of these operations, which were conducted exactly as stated in the last report of the referee[2], are as follows:

SAMPLE NO.	QUANTITY OF A B IN MIXTURE	QUANTITY OF O B IN MIXTURE	QUANTITY OF A B EXTRACTED	O B IN PETROLEUM ETHER	
	gram	*gram*	*gram*	*gram*	*per cent*
1	0.0040	.....	0.00368	......	....
2	....	0.0040	. .	0.00320	80
3	0.0024	0.0016	0.00231	0.00169	105.6
4	0.0008	0.0032	0.00110	0.00290	90.6
5	0.0032	0.0008	0.00324	0.00076	95
6	0.0020	0.0020	0.00196	0.00204	102
7	0.0036	0.0004	0.00366	0.00034	85

Recommendation 2, "that additional work be done on separating yellow A B and yellow O B from other oil-soluble dyes" was also not completed. However, a number of experiments were conducted by Scott to learn the behavior of other oil-soluble dyes when treated under the same condition. These experiments consisted of extracting 4 mg. of the dye in question with three portions of 40 cc. each of low-boiling petroleum ether in separatory funnels and extracting the solutions with an acid-alcohol mixture, as described in the previous report.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 74.
[2] *This Journal*, 1926, **9**: 362.

The results of these experiments are as follows:

DYE	QUANTITY EXTRACTED
Butter yellow, S & J No. 16	All
Aniline yellow, S & J No. 7	All
Sudan G, S & J No. 10	98 per cent
Sudan I, S & J No. 11	3 per cent
Sudan II, S & J No. 49	None
Carminaph garnet, S & J No. 60	None
Sudan brown, S & J No. 59	Small (extract a light blue)
Oil scarlet, S & J No. 150	Small
Sudan III, S & J No. 143	Trace

Work on Recommendation 3, "that the method of quantitative separation of amaranth from tartrazine be undertaken", was begun, but owing to incomplete data a report cannot be submitted at this time.

It is recommended[1] that the work planned for next year be a continuation of the previous work, with the object in view of bringing it to a definite conclusion.

REPORT ON METALS IN FOODS.

By W. F. Clarke (Bureau of Chemistry, Washington, D. C.),
Referee.

ARSENIC.

Although not recommended for study, arsenic was made the subject of collaborative work for three reasons: (1) the present situation in the fruit and vegetable industries with reference to spray residues; (2) certain features of the official method which should be more exactly defined; and (3) the necessity for establishing the limit of accuracy of the method.

For this collaborative study it was decided to use the modification of the official Gutzeit method which was developed at the Baltimore Station of the Bureau of Chemistry, because this method has been used widely in work on spray residues. In its essential features, this modification differs little from the official method. Aside from variances in the wet combustion, the dilution of the digest, and the size of aliquot taken (with none of which the present study is concerned), the differences are as follows: (1) the use, in the Gutzeit generator, of 5 cc. of concentrated sulfuric acid instead of 20 cc. of dilute sulfuric acid (1 + 4) or of 20 cc.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 74.

of dilute hydrochloric acid (1 + 3); (2) the use of 5 cc. of potassium iodide (15 per cent) instead of 4 cc. of potassium iodide (20 per cent); (3) specific directions for heating instead of directing to "Heat to 90°C. * * * and heat for 10 minutes"; and (4) the procedure for cooling to 17°–19°C. instead of to 5°C.

The actual determination is made as follows: Using the apparatus described in the official method, the solution in the generator is adjusted to a volume of 30 cc.; 5 cc. of concentrated sulfuric acid is added and, after shaking, 5 cc. of potassium iodide (15 per cent). The generator is placed in a round pan of warm water (40°–50°C.) over a Bunsen burner, the bath is heated quickly to 90°C., and then the gas is turned off. During the heating care is taken to prevent the flame from playing directly under the generator. Three drops of stannous chloride (40 per cent in concentrated hydrochloric acid) are added and, after 5 minutes, the temperature of the solution in the generator is reduced quickly to a

TABLE 1.

Results obtained by the collaborators.
(Expressed as micrograms of As_2O_3.)

	1	2	3	4	5	6
C. A. Greenleaf	14	16	15	17	22	21
	16	17	22	16	20	18
	15	14	18	19	16	20
	16	15	16	13	22	20
Average	15.3	15.5	17.8	16.3	20.0	19.8
G. W. Kirby	16	17	20	18	22	18
	20	18	18	18	22	20
	16	17	20	18	20	20
	16	15	20	18	22	20
Average	17.0	16.8	19.5	18.0	21.5	19.5
T. F. Pappe	19	20	21	20	22	19
	18	20	21	20	24	23
Average	18.5	20.0	21.0	20.0	23.0	21.0
H. R. Smith	18	25	21	20	25	25
	18	13	24	23	25	23
	14	14	16	19	16	17
	17	17	22	17	23	17
Average	16.8	17.3	20.8	19.8	22.3	20.5
W. F. Clarke	18	20	17	17	20	21
	17	16	17	20	19	17
Average	17.5	18.0	17.0	18.5	19.5	19.0
Grand Average	17.0	17.5	19.2	18.5	21.3	20.0
Present	16.5	17.4	18.3	19.2	20.1	21.0

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[1] As explained later the zinc should be sensitized and uniformly etched.

range of from 17° to 19°C. Cooling is accomplished readily by several substitutions of ice water. About 15 grams of stick zinc[1] is added to the solution, and the determination is completed as in the official method, except that the temperature is kept at 17°–19°C. instead of at 5°C.

It was considered that the essential point was to establish the limit of accuracy of the method. Consequently, the samples used were solutions of arsenious acid in dilute sulfuric acid, free of organic matter, so that no wet combustion was necessary. The six samples prepared were so varied in strength that the aliquots which the collaborators were instructed to use differed in a gradation of 0.9 mmg. from 16.5 mmg. to 21.0 mmg.

Each analyst obtained results that were in error, in at least one instance, by as much as 3.5 mmg. The averages were generally not in error more than 2.5 mmg.

DISCUSSION.

Although the official method does not direct the use of treated zinc, the usual practice is to use about 7.5 grams of new zinc and 7.5 grams of zinc which has been left from previous determinations. In the present instance, G. W. Kirby, C. A. Greenleaf, and the referee used zinc which had been treated in the following way: about 200 grams of the broken stick zinc was treated with 300 cc. of dilute sulfuric acid to which had been added 2 cc. of the stannous chloride solution; the action was allowed to continue 15 minutes; and the zinc was then washed in a funnel with tap water and finally with distilled water. From the results obtained by these three collaborators it was apparent that uniformly etched, non-pitted zinc was essential. The width of the mercuric bromide paper must be uniform. Wherever practicable, standards should be run along with the samples under examination, and in the present case those which were run simultaneously with the samples corresponded to 15 mmg., 20 mmg., and 25 mmg., respectively, of arsenic trioxide.

As frequently used, the Gutzeit methods involve the taking of very small aliquots; for such work a reliable volumetric method might prove suitable, permitting the use of the entire amount available. In this connection attention is directed to the official method for total arsenic[1].

LEAD.

The work on lead was disappointing, owing, probably, to the fact that samples were chosen with the intention of covering the worst case. Certain foods, e. g., oysters, may run as high as 2500 p. p. m. of copper and 3100 p. p. m. of zinc. To cover these possibilities, the samples were prepared with the following metal content: 0.3 gram of copper, 0.4 gram of zinc, and 0.1 gram each of tin, aluminum, and iron. The lead content ranged from 0.01 to 0.08 mg. The results obtained in preliminary work were so erratic that it was decided not to submit them for collaborative work.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 47-49.

In view of the results obtained last year, it is felt that further study is justified, and that, if found necessary, a less sophisticated mixture be used.

COPPER AND ZINC.

Since no method for either copper or zinc which seemed especially desirable was reported, no work was carried out on these metals.

RECOMMENDATIONS[1].

It is recommended—

(1) That the Gutzeit method (official and modifications thereof), and the most promising gravimetric and volumetric methods be studied collaboratively with a view to defining more exactly the conditions of the first and to offering the second and third, or one of them, alternatively with the official method.

(2) That the sulfocyanate method for lead be studied further.

(3) That methods be sought for copper and zinc.

No report on zinc in dried eggs was given by the associate referee.

REPORT ON FRUITS AND FRUIT PRODUCTS.

By H. J. WICHMANN (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Referee.*

Three reports were submitted by associate referees on subjects included under the designation of Fruits and Fruit Products:

1. Report on Water in Grape Juice, by B. G. Hartmann.
2. Report on Fruit Acids, by E. K. Nelson.
3. Report on Ash in Fruit Products, by H. J. Wichmann and Doris H. Tilden.

Hartmann's report indicates that the method he has developed is sound in principle and will, with careful work, determine approximately the percentage of added water in filtered white grape juices such as are ordinarily found on the market. Low results were obtained with unfiltered juices, unless the actual solubility coefficient for cream of tartar of the juice could be determined. Since it is the customary trade practice to filter the storage juice before bottling, unfiltered juices will hardly be found in interstate commerce. Further work appears unnecessary.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 74.

It was a disappointment to the referee that Nelson was unable to report a workable method for the gravimetric determination of active or inactive malic acid in fruit products. He has felt the necessity for such a method in the analysis of fruit products ever since the inactive malic acid became a commercial article. Like Nelson, the general referee has been able to convert pure malic into fumaric acid with better than 90 per cent efficiency, but he has obtained no satisfactory results on malic acid extracted from fruits. He agrees with Nelson that if a gravimetric method for malic acid is found it will involve its conversion into fumaric acid. The necessity for a method that is not dependent on optical properties is so urgent that further work is justified, and it is so recommended.

The referee is convinced that ash analysis, or rather the determination of the inorganic constituents of foods, is of great importance to agricultural chemists in general and especially so to those having to deal with fruit products. The interpretation of many analyses of fruit products depends in part on this determination. Naturally, ash material can be added in sophisticated products, but it is not so easy to add the ingredients of the ash in the proper proportions. At the San Francisco Station particular interest has been shown in the inorganic composition of authentic samples of fruits, and the methods for calcium and magnesium reported by the associate referees have been used with excellent results. The methods are based on principles not found in most textbooks on quantitative analysis. The method for calcium dates back to the work of the Referee on the Detection of Neutralizers in Dairy Products[1]. It was necessary, however, to modify it to include magnesium. It is believed that iron and aluminum can be determined as the phosphates in dilute acetic acid, but the development of a method has not yet been attempted. Iron, aluminum, and manganese have merely been completely removed from solutions previous to the determination of calcium and magnesium. The development of methods for these elements, as well as a collaborative trial on a synthetic solution containing iron, aluminum, manganese, calcium, magnesium, potassium, and phosphorus is recommended to the next associate referee[2].

The question of the determination of chlorine has been considered often at the San Francisco Station, and the analysts have some data on the determination of chlorine in fruit products, but they are not in shape for publication at this time. The conclusion based on the work done so far is that the present method for the determination of chlorine in the ash is neither accurate nor satisfactory. This was previously pointed out by Browne and Gamble[3]. In this laboratory it has been found that the direct method as recommended by them is satisfactory in the case of a limited number of fruit products, for example, tomato juice, but that the chlorine is precipitated as a colloidal silver chloride that is not completely retained by the Gooch crucibles from most fruit juices. In the authentic fruit work all the chlorine data were obtained after ashing the juice in the presence of excess sodium carbonate. Data on this method of preparation for chlorine determinations will be ready for publication next year.

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[1] U. S. Dept. Agr. Bull. No. 524.
[2] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 75.
[3] *Facts about Sugar*, 1923, **17**: 552.

REPORT ON WATER IN GRAPE JUICE.

By B. G. HARTMANN (Bureau of Chemistry, Washington, D. C.),
Associate Referee.

The results of the collaborative study of the tentative method for the determination of added water in grape juice reported in 1925 were unsatisfactory. It is believed that the poor agreement obtained at that time was due to inadequate temperature control during the treatment with cream of tartar. A provision, which it was hoped would permit of better control, was written into the text, and the method was again submitted for study during the present year. The results reported by the five collaborators who participated in the work are far from satisfactory; in fact, they are not much better than those reported in 1925.

After a careful review of the reported data the conclusion was reached that the erratic behavior of the method was due to the unfiltered condition of the juice used for the preparation of the samples. The purity of the juice had been established. However, it was learned that the manufacturer, from whom it had been secured, had not subjected it to the customary trade practice of filtering before bottling. An unfiltered storage juice contains finely suspended cream of tartar which, if not removed, will redissolve at a higher temperature, thereby increasing the cream of tartar content over that normally present in a factory-filtered juice. A thorough examination showed that the juice had a solubility coefficient for cream of tartar of only 0.052 gram per 100 cc. at a temperature of 25°C. Since, as has been shown, a filtered factory juice requires 0.095 gram of cream of tartar per 100 cc. for complete saturation at 25°C., it is at once evident why the results reported by the collaborators are low.

In the following table the collaborators' original results, based upon the factor 0.095, are given, as are the percentages of added water obtained by using the saturation factor 0.052.

In addition to the data given in the table, reports were also received from two other collaborators. Their results are not included in the table, however, because they are wholly out of agreement with the true amounts of water added, being in some instances four times too high. No positive

Percentage of added water found in five samples submitted to collaborators.

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
	2.5% Added Water		5.0% Added Water		9.8% Added Water		24.3% Added Water		54 0% Added Water	
	Factor, 0 095	Factor, 0.052	Factor, 0 095	Factor, 0 052	Factor, 0.095	Factor, 0 052	Factor, 0.095	Factor, 0 052	Factor, 0 095	Factor, 0 052
L. Jones Chicago, Ill.	−2.9	3.5	1.6	8.8	3.4	10.6	15.9	22.3	41.7	48.9
	−2.9	3.5	1.2	8.4	3.4	10.6	15.9	22.3	43.3	50.4
H. L. Clapp Chicago, Ill.	−2.8	4.4	1.6	8.8	4.4	11.6	13.8	21.0	40.8	48.0
	−2.2	5.0	−0.6	6.6	7.6	14.7	12.3	19.4	41.0	48.1
F. Hillig, Washington, D. C.	−5.3	2.7	−2.1	5.9	2.0	9.2	17.1	24.2	40.9	48.0
	−5.3	2.7	−2.1	5.9	2.0	9.2	15.5	22.7	42 1	49.3
B. G. Hartmann	−6.4	0.8	−1.7	5.4	6.0	13.2	15.0	22.0	38.9	46.1
Average		3.2		7.1		11.3		22.0		48.4

conclusion as to the reason for the discordant results obtained by these analysts can be offered. It may be assumed from the accuracy obtained in the titration of the five solutions that the method used was satisfactory, which supposition would justify the conclusion that the failure to obtain better agreement was not due to a lack of uniformity in the samples submitted. The only plausible explanation for the extremely high results reported is that the cream of tartar used was not pure.

Referring to the table, it is seen that the results reported by the four analysts when using the 0.095 factor are quite uniform, although extremely low. When calculating the percentages of added water on the basis of the true solubility coefficient, 0.052, the results agree very well with the amounts of water added.

Based upon the results reported, it may be concluded that the revised method for the determination of added water in grape juice described previously by the associate referee[1] is sound in principle. It is, however, applicable only to factory-filtered juices, that is, juices resulting from the filtration of storage juices at storage temperature (approximately 15°C.).

Judging from long experience with the method, in its application to regulatory work, and considering the good agreement had by the four analysts when using the 0.052 factor, the associate referee assumes that the fundamental principles of the method are sound. Extreme accuracy cannot be expected since each 0.1 cc. of 0.1 *N* sodium hydroxide corresponds to 3.1 per cent of added water. It is believed that the method in its present form is satisfactory and does not require further attention[2].

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[1] *This Journal*, 1926, 9: 38.

[2] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 75.

REPORT ON FRUIT ACIDS.

By E. K. NELSON (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

During the past year the work on the determination of fruit acids has been restricted to an attempt to develop a gravimetric method for the estimation of malic acid, either active or inactive.

The method used followed the lines suggested by the associate referee at the last meeting, namely, extraction of the total fruit acids by ether, conversion of the malic acid into fumaric acid by heating the sodium salts with an excess of sodium hydroxide at 130°C. for 3 hours, extraction of the fumaric acid, and oxidation to racemic acid by heating with a solution of 3 grams of sodium chlorate and 1 cc. of a 1 per cent solution of osmium tetroxide, the racemic acid being finally weighed as the difficultly soluble calcium salt.

While conditions were found whereby a yield of 90 per cent of fumaric acid could be obtained from pure malic acid, and a yield of 96 per cent of racemic acid from fumaric acid, the method has failed so far to give reliable results on acids extracted from fruit juices for the following reasons: (1) The long extraction necessary is quite impractical; (2) it has not been found possible to completely free the fumaric acid formed from the dehydration products of citric and tartaric acids, thus getting a satisfactory blank; (3) when operating on the acids as extracted from fruit juices, the step involving the oxidation of fumaric acid to racemic acid is interfered with by something that seems to poison the catalyst, and the reaction is no longer 96 per cent complete, but far below that figure.

Some collaborative work was undertaken in which the weight of malic acid determined from the weight of fumaric acid was requested, but in which the request for oxidation to racemic acid was omitted. Only one report was received.

The samples sent out consisted of: I.—Black raspberry juice, depectinized, with no added acids; II.—the same juice with 1 gram of inactive malic acid and 1 gram of tartaric acid per liter added; and III.—the same juice with 5 grams each of inactive malic acid and tartaric acid per liter added.

L. J. Cross of the Department of Farms and Markets, Albany, N. Y., reported the following quantities of malic acid expressed as grams per liter: I, 0.028; II, 0.448; III, 2.208.

These results are over 50 per cent low, the deficiency being due mainly to the fact that, while the ether extraction was carried out for the specified length of time, the rate of extraction, as shown by the volume of the aqueous solution remaining in the extractor, was much too slow.

It is unnecessary at this time to record the many experiments carried out by the associate referee. Until a practical reliable procedure, with the difficulties ironed out, can be found, no collaborative work will be attempted.

The results, however, seem to justify further work and this is recommended[1].

REPORT ON ASH IN FRUIT PRODUCTS.

By H. J. Wichmann, *Associate Referee*, and Doris Tilden, *Analyst* (U. S. Food and Drug Inspection Station, San Francisco, Calif.).

That plants contain the elements iron, aluminum, manganese, sulfur, chlorine, and silicon, but usually in small quantities, and calcium, magnesium, sodium, potassium and phosphorus often in relatively large quantities, is well known for the reason that these elements are found in combination in ash of plant material. Therefore, the analysis of plant ashes has been considered important in the past and, in the opinion of the associate referee, will be of greater importance to agricultural chemists in the future.

The methods of analysis in use at the present time have not been entirely satisfactory. In the case of fruit products iron, aluminum, manganese, silicon, and sodium are usually not determined, being mostly of minor importance except for the difficulties they create in the determination of the other elements, particularly calcium and magnesium. The time-honored methods require the removal of iron, aluminum, and phosphorus by the basic ferric acetate method before the other metals can be determined. In the presence of large quantities of phosphates, alkaline earths, and alkalis this method is not only cumbersome, but it is likely to be inaccurate. *Methods of Analysis*[2] contains methods for the determination of calcium and magnesium that do not require the previous removal of iron, aluminum, and phosphorus. These methods, however, require double precipitations and long standing, and they do not make any allowance for the presence of manganese, a consistent, if small, constituent of plant ashes[3]. If manganese is present, it will be found as a contamination of the magnesium phosphate precipitate. In the opinion of the referee and his associates, iron, aluminum, and manganese should be removed from solution before the precipitations of calcium and magnesium are made, but this procedure is not necessary with phosphates if a method developed by the referee is used.

This new method is a departure from old analytical methods. It is based on the insolubility of ferric and aluminum phosphate and the solubility of manganese, calcium, magnesium, and alkali phosphates in dilute acetic acid solutions. Manganese is separated in acetic acid solution as hydrated manganese dioxide by bromine water in the presence of sodium acetate. The calcium is then precipitated as the oxalate, with sodium oxalate. In this way calcium can be determined accurately and separated from magnesium in solutions containing phosphates. Ammonia and ammonium salts are purposely avoided up to this point because they are unnecessary and must be removed before the magnesium precipitation. The filtrate from the calcium oxalate contains the alkalis and magnesium, and the magnesium can be precipitated as the phosphate by the usual methods after the acetic acid has been neutralized.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 75.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 41, 42.
[3] J. S. McHargue. *Ind. Eng. Chem.*, 1926, **18**: 172

Ferric and aluminum phosphates are completely precipitated at a hydrogen-ion concentration of 3.0 to 3.5, but calcium and magnesium phosphates are not precipitated below a concentration of 7.0[1]. Calcium is precipitated in the presence of phosphates as the oxalate without contamination with phosphates and magnesium at a hydrogen-ion concentration of approximately 4.6[2]. Dilute acetic acid is therefore used to control the hydrogen-ion concentration.

In the statement of the principles governing the new method it will be observed that sodium oxalate was recommended as the calcium precipitant. This selection was made for the purpose of avoiding the introduction of ammonium salts previous to the magnesium precipitation. Unfortunately, it was found in check experiments that the results for calcium were uniformly slightly high by either gravimetric or volumetric methods, although the amount of magnesium determined in the filtrate was equal to the quantities added. This condition was attributed to occlusion of sodium oxalate in the calcium oxalate precipitate. The calcium, therefore, was dissolved in hydrochloric acid and reprecipitated with ammonium oxalate, and then determined gravimetrically, or volumetrically with potassium permanganate, as preferred. If the determination of magnesium is not desired, the preliminary precipitation of calcium with sodium oxalate can be omitted. In such cases there is no necessity for the avoidance of ammonium salts, and one precipitation with ammonium oxalate is ordinarily sufficient.

The new method recommended for trial by the referee for calcium and magnesium in the ash of plant materials is as follows:

CALCIUM.

Dissolve the ash in hydrochloric acid, evaporate to dryness, and bake at 110°C. for 1 hour to dehydrate the silica. Dissolve the residue in dilute hydrochloric acid and filter from any silicon dioxide. Make the filtrate up to a definite volume and take aliquots. To the aliquot taken for the determination of calcium and magnesium add a few drops of concentrated nitric acid and boil for a few minutes to oxidize all ferrous iron to the ferric state and to boil off any nitrogen dioxide. Make alkaline to methyl orange by adding pure 10 per cent sodium hydroxide (do not use an old solution) drop by drop while stirring. Add acetic acid to make the solution acid (not over 1 cc. of concentrated acetic acid in excess). Boil and add a few drops of sodium dihydrogen phosphate solution to provide an excess of phosphoric acid in all cases. Filter from any precipitate of ferric and aluminum phosphates and wash carefully. Only in exceptional cases will it be necessary to dissolve and reprecipitate the iron and aluminum phosphates.

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[1] *This Journal*, 1923, 6: 418.
[2] Alfred Shohl. *J. Biol. Chem.*, 1922, 50: 527.

Add 2 grams of sodium acetate and sufficient bromine water to color the solution yellow. Cover with a watch glass, bring to a boil, and boil briskly for a few minutes. If any manganese is present it will be precipitated as the hydrated manganese dioxide. (In fruit products the quantity of manganese is so small that it may usually be neglected. In other products, like seeds and spices, it may be quite appreciable.)

Filter the manganese dioxide and wash with hot water. If the precipitate of manganese dioxide is bulky (in excess of 10 mg.), dissolve it in a small quantity of strong hydrochloric acid and then reprecipitate it. Add the second filtrate to the first, boil off any bromine remaining, and evaporate the filtrate to 100–150 cc. Bring to a boil and precipitate the calcium by adding saturated sodium oxalate solution drop by drop to a slight excess. Continue boiling until the calcium oxalate begins to settle, or digest for 15 minutes on the steam bath. Allow to settle until the solution is clear (usually not over 30 minutes is necessary). Filter off the calcium oxalate and wash thoroughly with hot water. Reserve the filtrate for the magnesium determination. Wash the precipitate carefully back into the original beaker, heat, and dissolve it in as little concentrated hydrochloric acid as possible. Add ammonia drop by drop with continual stirring till the solution is slightly ammoniacal to methyl red. Then add acetic acid till slightly acid and while still hot add a slight excess of ammonium oxalate (only a few drops will be necessary to provide an excess of oxalate). Digest on the steam bath for 1 hour and set aside until the precipitate settles clear, or overnight. Determine the calcium either gravimetrically or volumetrically by the usual methods. For small quantities of calcium the gravimetric method is preferred.

If magnesium is not to be determined, precipitate the calcium once from the boiling solution freed from iron, aluminum, and manganese with ammonium oxalate; digest; and determine as described.

MAGNESIUM.

If large quantities of calcium have been precipitated, use both filtrates. Concentrate the filtrate from the first calcium precipitation to 50–75 cc. Make the solution slightly alkaline to methyl red with ammonium hydroxide. Heat to boiling and add sodium ammonium phosphate solution drop by drop to slight excess. Add one-third the volume of concentrated ammonium hydroxide slowly with constant stirring. Let stand for 2 hours, or overnight. Filter, wash the precipitate with water containing 2 per cent of ammonia till all chlorides have been removed, dry the precipitate, and ignite in a platinum crucible at first gently and then with a blast. Weigh the magnesium pyrophosphate and calculate to magnesium oxide.

The experimental work in connection with the development of this method was done on the purest magnesium ribbon obtainable and ignited calcium oxide prepared from calcium oxalate precipitated at least two times. In many analyses, iron, aluminum, phosphates, and manganese in appropriate proportions were added to the initial calcium-magnesium solution. The numerous experiments made to check the details of the method need not be given here. Some of the results obtained by the junior writer on the final method are given in the following table.

CaO PRESENT *gram*	MgO PRESENT *gram*	CaO FOUND *gram*	MgO FOUND *gram*
	0.0575		0.0579
0.0922	0.0751	0.0919	0.0755
		0.0929	0.0756
0.0964	0.0760	0.0964	0.0768
0.0711	0.0760	0.0774	0.0761
0.0541	0.0772	0.0548	0.0772
	0.0772		0.0775
0.0906	0.0772	0.0906	0.0764
0.0272	0.0232	0.0275	0.0237
		0.0275	0.0236

Calcium oxide determined volumetrically with 0.1 N potassium permanganate.

0.0906	0.0910
	0.0909
	0.0907

This method has given consistently good results in the hands of the referee and his associates, although it has not yet been put to the test by collaborators working under conditions perhaps entirely different from those of the writers. It is felt, however, that it has decided merits, and it is offered for cooperative trial by the next associate referee[1].

No report on canned foods was given by the referee.

ADDRESS BY DR. WILEY.

LUX ET VERITAS.

I wish to assure you, Mr. President, that I am not responsible for the title of my paper. This is the first time I have ever addressed this organization since I have been honorary president that I have been fettered by a title to my address. Heretofore I have been permitted to choose my own theme and to feel no restriction. Well, I think I ought to confine myself to my theme, but it is so limiting and means so little—even though the motto of a somewhat renowned university—that it gives me a very restricted opportunity for originality.

We have had opportunities to look at the sun, at least through a smoked glass, and yet we all realize that no one has ever seen the sun in its proper position. Do you realize that? The sun rises eight minutes before we can see it at all, and it shines eight minutes after it has set.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 75.

That is a peculiarity of light—that it is not transmitted instantaneously. It takes eight minutes for the light to come from the sun to the earth. Of course I was taught, and you were too, that the earth was the first creation and that the sun and moon and stars were made for our delectation. Well, they have been delighting us for a number of years in various ways, but now we have applied the test of research to these heavenly bodies and have discovered the most marvelous effects, some of which I will relate to you.

I have just had the opportunity of reading about the oldest light that is known—a light that started on its way to this planet 10,000,000 years ago. I suppose it was meant for us, because it has come to us now. The study of this light was not concerned with what it may be now, but rather as to what it was at its source, 10,000,000 years ago. Now, that is the most remarkable piece of light that I have ever read about. It so happened that my wife's niece, Adelaide Ames, was one of the persons who led us to this distant world. She and Professor Shapley, the Professor of Astronomy of Harvard University, have told us all about it in a most interesting paper. As that light has been traveling at the rate of 186,000 miles a second for 10,000,000 years, you may know it has come a long distance, and the source of it may have long passed away. You cannot look tonight, but last night if you had looked at the stars instead of at the boxing match you might have thought they were still there, for they were shining as they have shone ever since we were born. Some of these lights have been a million years on their road, and you saw something that was there a million years ago if you looked up at the heavens last night. You cannot see this new light without a special apparatus. I have seen the pictures of it, but that is all. But it is the light that comes from the most distant quarters of the universe, and yet it is the same kind of light that comes from the sun to us every eight minutes, and the light you see in the heavens is the same kind even if it has been 10,000,000 years on the way. What does that give us as the idea of the great cosmos? Unity, unity.

As we study them we can tell the chemical composition of these luminous bodies. What do we find? We find the things that we find on this earth, and more. What is the lesson which that conveys? A lesson of such grandeur, of such incomprehensible unity and plan that the human mind cannot conceive of it! What does research do? Dr. Browne says that it helps to regulate officials, but it does more than that —it reveals to us the secrets of the universe.

A beautiful illustration of my theme occurred to me many years ago in Berlin at the meeting of the International Congress of Applied Chemistry, when Mr. William Crookes, eminent chemist, was president of the association. On that occasion Dr. Otto Witt, another eminent chemist and the one who succeeded Dr. Crookes in the presidency, introduced him by the following well-known old church motto, which was most appropriate: "Ubi Crookes ibi lux"—"Wherever Crookes is, there is light". How beautifully applied it was in that instance! Of course, Professor Crookes, like some other great men, is not always sane—I don't know of one that is always—for lately he got the idea that he could talk with disembodied spirits. Well, he may be able to do that for anything I know, but I never have had the privilege of talking with the spirits or of having anything to do with them. The only spirits I have had anything to do with were mostly in the bottle, and the less you talk about that kind of spirits the better it is for you. But that was one of the greatest compliments I had ever heard paid to a scientific man.

Now, the other part of my theme is not so encumbered: Veritas! Lux et veritas!

The word "veritas" is on the shield of Harvard University, but unfortunately *sine lux*, and has been now for 300 years, so I am quite familiar with it. When they built the Harvard stadium, which was the first of the college stadia, they wanted to follow the good old New England custom of using some inscription from the Bible, and so they asked different persons to suggest something appropriate. One man sent in—by the way this was not used, although everybody hoped it would be—this quotation from the Bible: "The sons of Eli are liars and there is no truth in them". On another occasion, speaking of the Harvard shield, dear old Dr. Peabody and Edward Everett Hale met on their way to Soldiers Field on a Saturday when Yale and Harvard were contesting. Someone said to Dr. Peabody: "Are you going out to shout, 'To hell with Yale?' " He answered: "No, I am going out to yell with Hale". You see *veritas* saving the day again.

There is a lot in those two words. I am almost glad that they were assigned to me by some unknown friend. I accosted the only real classicist that I have met since the death of my lamented friend, Professor Sophocles, years ago and accused him of perpetrating this, but Dr. Browne says he did not do it, so I am still unable to thank the one who did do it. (Since then I have learned that Dr. Skinner is the culprit.)

There is always opportunity to spread a little more light and stick a little closer to the truth. Have you ever noticed how differently people interpret the same data? You will find a rabid free-trader and an unreformed high tariff man form absolutely diametrical opinions on the same data. You will see one man walking up in his ignorance and superiority and voting the Democratic ticket, while another man with equal fixity of purpose votes under the same instructions in politics and selects the Republican ticket. Is it not strange? Now, both of these men are probably honest, and they are going in the way in which they think they ought to go, but they go in diametrically different ways. The same is equally true of economic and religious principles; we all have the same data at our disposal, but after study we reach very different conclusions. I think it is a happy thing that this is so. What a monotonous life this would be if everybody believed the same thing, voted the same way, pursued the same political party, belonged to the same church—there would be no use living in this world as the only refuge a man would have would be to get married. Perhaps then he would not have such unbroken monotony.

I have, as has been said today, attended every meeting of the Association of Official Agricultural Chemists, and as long as I am able and have the opportunity I shall continue to do so. I wish never to miss one of them as long as I live.

Now, what are some of the truths that we may use to help us to a longer life? I think we all would like to live as long as possible. Yesterday morning at the breakfast table, when we were discussing my 82nd birthday, my good wife said she hoped that I would live to be 92. She wanted me to live as long as President Eliot lived and he died young—that is the trouble with people, the good die young no matter how long they live. I should be perfectly content to look forward to another ten years if I could be as useful and as helpful in those ten years as Dr. Eliot was almost to the very last. He was most active in all good work and I never knew him to be engaged in any work except that kind. He was a young president when I went to Harvard—had only been there three years. He was in the making, and there was a great deal of doubt about his selection. It was only the second time that anyone except a minister of the Gospel had been chosen president of Harvard, and it was uncertain what was going to happen without an old man in the saddle. However, after forty years of that presidency, while still in the strength of his vigor and the vigor of his mentality, Dr. Elliot asked to lay aside the burden, not because he felt unable to do the work but because he wanted someone else taken in and trained up to do the job as well as he had done it. After he resigned from the presidency he was offered the highest position in public life. I remember when Dr. Eliot was here. It was at the beginning of Mr. Taft's administration, when we had a great Harvard gathering in his honor. We had two presidents at the dinner table, the President of the United States and the President of Harvard University. What an impression he made on that crowd of Harvard men by his dignity, by his reserve power, clearness of vision, his order of speech, his clarity of thought! No one but a young man could have exhibited all these characteristics of strength and youth. Mr. Taft urged and begged him to accept the post of Minister to the Court of St. James, but he declined because he thought that at his time of life he had better not begin any new career but stick to what he had been accustomed to do—work for the public good. Again, another president wanted to send him as minister to China and his Secretary of State objected because he thought he was not a Christian. He belonged, you know, to the Unitarian Church. Of course he would not have accepted the place if it had been offered to him.

So, there was a man who showed in his career, and especially the last years of his career, the Harvard principle of veritas—truth; truth not for any gain, but for truth's sake.

We can take this element of truth, apply it to research, and we can add to the length of human life on this sphere. We learn from astronomy what a speck, microscopically speaking, this world is. Even our sun is only a speck. It is only one of thousands of stars that make our own system, our own bunch of stars, the same ones that Professor Shapley looked at. Ten million years of light away and yet today it is the only thing in the world.

When we leave here we do not know where we are going. We believe in eternal life by tradition. We hope. But I think every reasonable human being wants to stay right here just as long as possible. This thought brings me back to the conversation at our breakfast table. My older boy said: "Why stop at 92? I want Daddy to live to be a hundred". Then my younger boy said: "What's the use of limiting Daddy's age? I want him to live as long as he wants to". There is the true philosophy. I would like to do that, but I don't want to live if I am unable to do anything to help along the cause of truth. Whenever I am unable to add anything to the ethics of living, to truth in its application to those ethics—truth applied to life; when I can do no more good; when I become a burden on any human being, either mentally or physically or otherwise, then my desire for life ends. However, we can keep that spirit of sacrifice and duty alive many, many years yet. Already, due to the application of research, the average length of human life in this country has been advanced about ten years or a little more, and statistics show that it is now about 54½ years for men and 56½ years for women. These figures show which sex nature believes in. The male is not considered by nature as of any value but the female is, and therefore women are more virile, you might say, than men.

As we increase this research and as we learn more about how to maintain youth and vigor, we will increase the average length of life. Perhaps in another 25 years we will increase it by three or four more years. Dr. Irving Fisher, at the Public Health Association the other day, even prophesied that before the end of the century the average length of human life would be 80 years. I think he is a little optimistic because as we improve in anything our improvement slows down. If we begin to play billiards and golf—as most people do nowadays—first we improve rapidly and then more slowly, and as we get to the end of our improvement it is very slow indeed. So, in adding to human life, at first we can do so rapidly, then we will have to slow down a bit, because the task becomes more severe and more difficult and we will not make the same progress towards the end of the century that we did 25 years ago; we won't make the same progress in the next 25 years that we did in the last. But research, the studying out of new truth and light, is one of my themes that is going to help us, just as during the last few years the therapeutic value of light has been brought out in a manner as never before, and has begun to be applied in the home. The sun worshipers have the only rational religion.

I had a most remarkable letter yesterday from a woman who never puts any clothes on her child in the house because she wants him to get the full benefit of light. If you expose a child with rickets to the light it is a good deal better than any medicine you can give him. Light, if not filtered through glass, is the most wonderful therapeutic aid to humanity. Light, and the heat that accompanies it, is the sole source of vitality on this planet, and therefore we must again turn back to my theme as the foundation on which to base our respect for light, because through light and heat we get our food and clothing, our books, and our machines—all the appliances which make life less burdensome. We get vitality through the same source, not only indirectly through foods, but directly through the light rays on our body. We shall see a great reform in our food and clothing, especially indoors—we do not need much reform outdoors now—but we will see more exposure to the light through windows of quartz glass. Our houses will not be built for external beauty alone—I hope there will always be external beauty, because I am a great believer in architecture—but they will be built also as sanitary places in which to live.

If you will study diseases you will find that most of them are house diseases anyway, especially tuberculosis. Therefore, as the means to increased longevity, we will live more outdoors, with less clothing and with more exposure to the sunlight as the therapeutic agent. The day will also come when not so many of us will have grown through our hair as we have today. I notice that nearly all these great orators I have listened to in the last few days have grown until they have perforated the hair. I am a little that way myself, but you don't see women that way. Why? Because unknowingly they are obeying the laws of sanitation and of sanitary science applied to hair, and they don't wear any tight bands around the head to shut off circulation from the scalp. They occasionally go to have a permanent wave put in, but that will last them, I am told, only three or four months—the Queen of Rumania has just had one, so you can ask her. But they don't go so often to the barber and get their scalps infected as men do; and then, too, men go and cut off all the food from the top of the head with the result that you can readily see. That does not help you to live longer, to lose your hair. I want to see an octogenarian with all the hair that is coming to him and all that ever did belong to him. I do not object to its getting white.

That is no shame or disgrace, but even that is due to some interference with the proper pigmentation of the hair in some way. Maybe we will be able to retain the natural color of the hair as well as the hair itself.

So, all the things which are wrapped up in this word "lux" are most interesting to us from every possible practical point of view.

Now, my friends, I am not going to detain you any longer. If I could follow this out I would keep you here for a long period of time. I can tell you, however, that I have found out the definition which I have long been seeking to suit my own case as a proprietor of a Virginia farm, where I get health and sunlight at least. I get them more now than I ever knew about eight or ten years ago. I stopped, practically stopped, wearing my hat. I know that these vitalizing rays of the sun, while they won't revive the hair that is dead, may keep alive what little remains alive. Perhaps I shall have another head of hair in a few years. But that is about all that I get out of my farm and so I was much rejoiced in running across this definition of a "gentleman farmer" the other day: "A gentleman farmer is one who raises his hat and nothing else". So, when I go out in the great open air and look up into these infinite heavens above me, in the presence only of that Great Being who has created this universe as a unity, even to 10,000,000 years of light away, I take off my hat and salute the great Author of Nature.

Chairman: Gentlemen, along with extending our thanks and congratulations to our honorary president for his address, I would be glad to extend to him our congratulations on his good health. Let us make an agreement with him now that we will meet him here in Washington, or wherever our next place of meeting is next year, and see how his scalp has developed. You will be there, Dr. Wiley?

Dr. Wiley: Yes.

SECOND DAY.

TUESDAY—AFTERNOON SESSION.

REPORT ON CEREAL FOODS.

By F. C. Blanck (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.), *Referee.*

Since the association took definite action at last year's meeting restricting the present year's activity to those methods already under active consideration, no new work was undertaken. While the general referee clearly recognizes the need for prompt and definite action on pending methods, sight must not be lost of the fact that chemistry is an advancing and changing science and that if the methods of the association are to maintain their merited position of excellence, reliability, and modernity, the members must be keenly alert to the current progress of this science.

The development of new and improved methods of analysis is always predicated on preliminary consideration of the problem, including the possible application of work already done in an allied field. Such advance consideration of the association's problems by the referees and associate referees should greatly facilitate prompt devising and trial of new methods and new determinations. Consequently, the general referee suggests that the various referees be encouraged to give constructive consideration to their assignments beyond the specific recommendations annually adopted.

There is appended to this report a selected bibliography of references in the cereal field, which may be of some interest. This bibliography only covers work that has been published since the 1925 meeting.

The referee takes this opportunity of expressing his appreciation of the splendid cooperation of the various associate referees and their collaborators. Without their careful, intelligent work, this report would not have been possible, as the referee is conscious of the fact that his own effort and thought have contributed but little to the progress here reported.

Special acknowledgment is also made of the fine cooperation accorded by the American Association of Cereal Chemists and the milling industry. Without this cooperation of men engaged in the chemical side of industry, the methods of the association will not attain that position of universal recognition which is so desirable.

SAMPLING OF FLOUR.

Further collaborative work on the method recommended by the associate referee[1] indicated the need for still further collaboration before its adoption as official.

MOISTURE IN WHEAT FLOUR AND IN ALIMENTARY PASTES.

Associate Referee G. C. Spencer subjected the routine air-oven method[2] to further collaborative study. A subdivision of the same sample of flour was sent to each of 19 analysts, together with instructions to make moisture determinations in triplicate on two successive days. The results were satisfactory and confirmed previous experience as to the reliability of this method.

ASH IN FLOUR AND GASOLINE COLOR VALUE.

Associate Referee D. A. Coleman, by means of collaborative study, showed that 550°C. is a sufficiently high temperature to use in ashing all classes of wheat flour providing the ash does not collect into a small area in the bottom of the ashing dish. Two methods of preventing this have been suggested, namely, the use of glycerol alcohol and of 60-mesh alundum. The alundum method for ashing consists essentially of mixing 4 grams of 60-mesh alundum with a 2 gram sample of flour in the ashing dish and igniting in the muffle at 550°C. This method appeared to give a whiter ash than the glycerol-alcohol treatment, and was therefore recommended for further study.

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[1] *This Journal*, 1926, **9**: 39.
[2] *Ibid.*, 40.

The glacial acetic acid method, which consists of ashing flour when mixed with a calcium acetate-glacial acetic acid solution, at a temperature of 900°C. proved unsatisfactory, and the associate referee recommended that further study of this method be dropped.

No work was accomplished on gasoline color value, and it is recommended that the studies be continued.

GLUTENIN IN WHEAT FLOUR.

Associate Referee M. J. Blish showed that 70 per cent boiling alcohol removes from the wheat flour, in addition to gliadin, some protein material from the glutenin. It appears to differ from glutenin and is doubtless a product split off by the boiling alcohol. Therefore, in view of Osborne's definitions of wheat proteins[1], which are based upon solubilities, it is suggested that the use of hot alcohol be prohibited in the quantitative estimation of wheat flour proteins.

Four methods for the determination of glutenin are available, two direct and two indirect, and all appear to give reasonably accurate results.

The method of Blish and Sandstedt[2] and the "barium hydroxide"[3] method are shorter and simpler than those proposed by Sharp and Gortner[4], and therefore they are recommended for collaborative study. The "barium hydroxide" method is based upon the observation that when two or three volumes of pure methyl alcohol are added to one volume of a solution of glutenin in barium hydroxide, the glutenin is precipitated. The protein determined in an aliquot of the supernatant liquid subtracted from the total protein of the sample gives the amount of glutenin.

THE HYDROGEN-ION CONCENTRATION OF FLOUR.

Associate Referee C. H. Bailey submitted for collaborative study a buffer solution, and also two samples of flour upon which collaborators were asked to determine the pH value. The values reported on the buffer solution were not in close agreement with the theoretical value and consequently some doubt is expressed as to the value of the pH readings obtained on the flour samples. In view of this discrepancy, the associate referee recommends that further collaborative work be done and that each collaborator standardize his set-up with the buffer solution before beginning the examination of the flour samples.

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[1] The Proteins of the Wheat Kernel. Carnegie Institution of Washington, 1907.
[2] *Cereal Chem.*, 1925, 2: 57.
[3] *Ibid.*, 1927, 4: 129.
[4] Minn. Agr. Expt. Sta. Tech. Bull. 19, 1923.

GLUTEN IN WHEAT FLOUR.

In washing glutens, Associate Referee C. B. Kress made use of the solution suggested by Dill and Alsberg[1]. It is a 0.1 per cent solution of equal parts of monopotassium phosphate and disodium phosphate. He reports that glutens obtained by the use of this solution are higher in yield but lower in quality than those obtained from ordinary tap water when the same sample of flour is used.

He recommends that further study be given to this determination and that salt solutions of various concentrations and hydrogen-ion concentrations be employed.

Experimentation thus far shows that ordinary tap water still remains the best electrolyte for washing glutens.

DIASTATIC VALUE OF FLOUR.

Associate Referee E. L. Tague submitted an effective plan for the development of a program of work on this determination, but no special report was submitted on account of the lateness of his appointment.

STARCH IN FLOUR.

O. S. Rask, the associate referee, developed a new and novel method for this determination. The collaborative data obtained are quite encouraging and warrant more extensive collaborative study during the coming year.

CHLORINE IN BLEACHED FLOUR.

Associate Referee G. C. Spencer continued the studies outlined by former Associate Referee Armin Seidenberg and found that the extraction of fat bodies from flour with hot 70 per cent alcohol is entirely satisfactory. However, the problem of the estimation of chlorine in the extract thus obtained still remains.

EXPERIMENTAL BAKING TESTS.

Associate Referee M. J. Blish secured the opinions of approximately 100 mill, bakery, consulting, and research wheat and flour chemists on the problem of test bakes. From this data he formulated the necessary requirements for a procedure. He proposes, first, to become acquainted with experimental baking procedures; second, to select a tentative method based upon the best features of all methods observed; and, third, to submit such a method to collaborative study.

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[1] *Cereal Chem.*, 1924, 1: 222.

UNSAPONIFIABLE MATTER AND FAT (ACID HYDROLYSIS) IN FLOUR AND IN ALIMENTARY PASTES.

Associate Referee Samuel Alfend submitted directions for collaborative study upon samples of flour, water noodles, and egg noodles. Two methods for the determination of the fat were used: the direct extraction and the acid hydrolysis. Collaborative results show that the latter method gives much higher results than the former and the associate referee, therefore, recommends the adoption of the acid hydrolysis method as official for flour and alimentary pastes.

Unsaponifiable matter was determined by two methods: the modified Kerr-Sorber and the F. A. C. Collaborative work shows that both methods give accurate results; however, the F. A. C. method is the most widely used and in the opinion of the associate referee should be adopted as tentative for flour and alimentary pastes. This determination should be made on the lipoids extracted by the tentative method as applied to egg noodles.

METHODS FOR BREAD ANALYSIS.

Associate Referee L. H. Bailey submitted for collaborative study on bread two methods for each of the following determinations: Total solids, ash, fat, and lipoids. Since only a limited number of collaborators responded, Bailey recommends that these studies be continued for another year. He also suggests that in the future consideration be given to the development of methods for determining the amounts of the characteristic constituent of special breads, such as the quantity of raisins in raisin bread, the quantity of rye in rye bread, and the quantity of milk in milk bread.

RECOMMENDATIONS[1].

CEREAL FOODS.

Flour.

It is recommended—

(1) That the method for sampling flour, adopted as tentative[2] last year, be subjected to collaborative study as suggested by the associate referee.

(2) That the present official vacuum oven method[3] for the determination of moisture in flour be dropped.

(3) That the vacuum oven method for the determination of total solids and moisture (indirect method) in flour[2], be adopted as official (final action).

(4) That the routine air-oven method for the determination of total solids and moisture (indirect method) in flour[4] be adopted as official (first action) and that the word "routine" be deleted from the title.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 78.
[2] *This Journal*, 1926, 9: 39, 89.
[3] *Methods of Analysis, A. O. A. C.*, 1925, 223.
[4] *This Journal*, 1926, 9: 40.

(5) That no associate referee on moisture in flour be designated for the coming year since these methods are now in satisfactory condition.

(6) That the associate referee continue studies on rapid methods for the determination of ash in flour, including the alundum method and the oxygen-acetate method[1].

(7) That the associate referee carefully study the nature and kind of losses occurring when ash is fused.

(8) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour[2], be adopted as an official method (final action).

(9) That the method for the determination of lipoids and lipoid phosphoric acid (P_2O_5) in flour[3], be adopted as official (final action).

(10) That the acid hydrolysis method for the determination of fat in flour[3] be adopted as an official method (first action).

(11) That the modified Kerr-Sorber method for the determination of unsaponifiable matter[4] be adopted as a tentative method for flour and subjected to further collaborative study.

(12) That the study of methods for the determination of glutenin in flour be continued and that the associate referee subject the Blish-Sandstedt[5] and "barium hydroxide"[6] methods to collaborative study.

(13) That the method suggested by the associate referee for the determination of the hydrogen-ion concentration of flour[7] be approved as a tentative method.

(14) That collaborative studies on the hydrogen-ion concentration of flour be continued during the next year, and that the collaborators be provided with standard buffer solution, to which their set-up shall be standardized before beginning the examination of flour samples.

(15) That the associate referee give attention to the possible use of the quinhydrone electrode in this connection.

(16) That the study of methods for the determination of gluten in flour be continued.

(17) That the study of methods for the determination of the diastatic value of flour be continued and that this investigation include: (a) a determination of the optimum hydrogen-ion concentration for the action of the diastase, (b) the influence of fermentation on this action, (c) the influence of proteolytic activity, and (d) methods.

(18) That work on the detection and estimation of flour-bleaching chemicals be continued with special attention to the determination of chlorine in chlorine-treated flours.

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[1] *Cereal Chem.*, 1926, 3: 222.
[2] *This Journal*, 1926, 9: 40, 89.
[3] *Ibid.*, 41, 89.
[4] *Ibid.*, 1925, 8: 441.
[5] *Cereal Chem.*, 1925, 2: 57.
[6] *Ibid.*, 1927, 4: 129.
[7] *This Journal*, 1927, 10: 33.

(19) That the study of methods for the determination of starch in flour be continued and that this study include the method proposed by the associate referee and also the recently proposed modification of the diastase method as suggested by Hartmann and Hillig[1].

(20) That consideration be given to the factors for the conversion of the percentages of nitrogen into terms of protein in wheat, wheat bran, wheat endosperm, and wheat embryo as suggested by Jones[2].

BAKED CEREAL PRODUCTS.

It is recommended—

(1) That collaborative study of the tentative method for the preparation of sample of bread[3] be continued.

(2) That the tentative method for the determination of total solids of an entire loaf of bread[3] be further studied.

(3) That the tentative method for the determination of total solids of the air-dried ground sample[3] be further studied.

(4) That studies of the 130°C. air-oven[4] and other rapid methods for the determination of total solids in an entire loaf of bread be continued.

(5) That the method for the determination of ash in baked cereal products[5] be adopted as official (final action).

(6) That the method for the determination of protein in baked cereal products[6] be adopted as official (final action).

(7) That comparative studies of the methods for the determinations of lipoids (as directed for alimentary pastes)[4] and of fat[7] in bread be continued.

(8) That the study of methods for the carrying out of experimental test bakes be continued.

ALIMENTARY PASTES.

It is recommended—

(1) That the tentative method for the determination of total solids and moisture (indirect method)[8] be studied collaboratively.

(2) That the study of the air-oven method for the determination of total solids in alimentary pastes[4] be continued.

(3) That the method for the determination of ash in alimentary pastes[9] be made official (final action).

(4) That the method for the determination of chlorides in ash as sodium chloride[9] be made official (final action).

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[1] *This Journal*, 1926, 9: 482.
[2] *Cereal Chem.*, 1926, 3: 194.
[3] *This Journal*, 1926, 9: 42.
[4] *Ibid.*, 40.
[5] *Ibid.*, 42; *Methods of Analysis, A. O. A. C.*, 1925, 225.
[6] *This Journal*, 1926, 9: 42.
[7] *Ibid.*, 41.
[8] *Ibid.*, 43.
[9] *Methods of Analysis, A. O. A. C.*, 1925, 232.

(5) That the method for the determination of organic and ammoniacal nitrogen in alimentary pastes[1] be made official (final action).

(6) That the method for the determination of protein in alimentary pastes[2] be adopted as official (final action).

(7) That the method for the extraction and identification of added color in alimentary pastes[3] be made official (final action).

(8) That the acid hydrolysis method for the determination of fat in alimentary pastes[4] be adopted as tentative and subjected to further collaborative study.

(9) That the method for the determination of lipoids and lipoid phosphoric acid (P_2O_5)[5] be studied collaboratively.

(10) That the modified Kerr-Sorber method for the determination of unsaponifiable matter[6] be adopted as a tentative method for alimentary pastes and subjected to further collaborative study.

(11) That during the coming year the associate referee study the application of the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour and alimentary pastes[5].

(12) That the tentative method for taking and preparing an analyst's sample of alimentary paste[7] be studied collaboratively.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 232.
[2] *This Journal*, 1926, **9**: 44.
[3] *Methods of Analysis, A. O. A. C.*, 1925, 233.
[4] *This Journal*, 1926, **9**: 41.
[5] *Ibid.*, 40.
[6] *Ibid.*, 1925, **8**: 441.
[7] *Ibid.*, 1926, **9**: 43.

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REPORT ON SAMPLING OF FLOUR.

By H. RUNKEL (U. S. Food and Drug Inspection Station, Minneapolis, Minn.), *Associate Referee.*

The previous report of the associate referee indicated that the work for this year should include further efforts to obtain comments of trade associations, food officials, and others interested in the tentative method as submitted, and that some collaborative data should be secured to furnish an adequate basis for a judgment as to the proper value of the method. The work was carried on in cooperation with the same committee mentioned in last year's report, namely:

M. A. Gray, Chief Chemist, Pillsbury Flour Mills Co., Minneapolis, Minn., representing Millers' National Federation;

Leslie R. Olsen, Chief Chemist, International Milling Co., Minneapolis, Minn.; and

D. A. Coleman, U. S. Bureau of Agricultural Economics, Washington, D. C., both representing American Association of Cereal Chemists; and

C. B. Morison, Assistant Director, American Institute of Baking, Chicago, Ill.

Grateful acknowledgment is made to the members of this committee for their whole-hearted assistance in planning and in carrying out experimental work. It is through them that the associate referee was able to secure the valuable viewpoints of the trade and the trade associations which these members represent.

The method was given as much publicity as possible. It was printed in News Letter No. 3 of the American Association of Cereal Chemists, which reaches the various members of this association; it was included in the 1925 report of the secretary of the Millers National Federation, which resulted in its distribution to a number of trade papers; it was printed in Baking Technology, the trade publication of the American Institute of Baking; and it was circulated to all the stations and some members of the U. S. Bureau of Chemistry, and to the various State food and drug officials who might be interested.

The character of the criticisms received is illustrated by the following comments taken from letters:

A. E. Paul, U. S. Food and Drug Inspection Station, Chicago, Ill.—
Date: February 4, 1926.

I believe that your general plan is sound and very desirable, but I feel that your reference to the various degrees of exposure could be made somewhat clearer The wording used might apply to sacks of which, respectively, three, two, one, or no sides are exposed; again, it might refer to the outer layer, the second, third, and fourth layers of sacks in the pile; or it might mean that the number of sacks mentioned should be taken from four zones varying equally from the surface to the center of the pile. The latter, I presume, is the interpretation intended by you, but it would seem that it might well be made very clear as to just what the intention is. It would seem, also, that there should be some mention made as to whether in the same zone contiguous sacks are to be taken, or whether the sacks should be taken at different points, and finally, whether units from different zones should be taken directly underneath each other, or whether they should be taken at different points.

J. O. Clark, U. S. Food and Drug Inspection Station, Savannah, Ga.—
Date: February 8, 1926.

We would suggest that the proposed method be modified to allow compositing the cores so that the number of subdivisions from each shipment would be materially reduced. It might be wise to composite the cores from all outside sacks and make another composite from all inside sacks, or, again, it might be satisfactory to composite all of the cores as is done at the present time.

Date: March 8, 1926.

As the method is written, I do not think that the last paragraph would permit compositing for the moisture determination. Why not change this paragraph to read as follows?

"For such determinations as moisture, net weight, uniformity, and baking tests, samples may be increased in number, increased in quantity, or combined to suit the requirements of the analyst if the principles laid down above are followed".

C. S. Brinton, U. S. Food and Drug Inspection Station, Philadelphia, Pa.—
Date: February 11, 1926.

Would it not be satisfactory for the samples to be drawn in the way you have mentioned from a large number of sacks and the composite sample analyzed for moisture content instead of running each one separately? Would it not be more advisable to sample the various sacks according to their exposure, as mentioned in your second paragraph? This would reduce the number of samples to be actually analyzed to only four, which would not be unreasonable, and this procedure would be uniform then for any size shipment.

R. E. Doolittle, Central Inspection District, Chicago, Ill.—
Date: February 16, 1926.

We must appreciate that if it is not possible to have a method which can be demonstrated theoretically and practically as scientifically accurate, the more simple, if approximately correct, it can be made, the more generally it will be followed. Perhaps in this method it would be well to determine whether or not, in the opinion of those who have to do with the sampling of flour, the method, if adopted, would be followed.

The directions, with the exception of paragraph 2, in my opinion are splendidly given. I do not see how we can expect with these directions that two samplers would obtain flour of the same composition, if exposure has anything to do with composition. It is easier, however, to criticise adversely than it is to give suggestions for overcoming objections and I must admit that I do not know just how to overcome the indefiniteness which appears to me to be present in this paragraph. Without going into too much detail, I do think that if it is necessary to include something of this kind, it will be better to direct the particular sacks to be sampled and where; for instance, by inserting the trier in the upper surface of each of four bags on the top of the pile, in the end of the next to the top of the pile, etc.

What I should like to see are directions that would insure that if I sent down and sampled a lot of flour you or someone else had sampled, we would have sampled the same bags in the same way. These directions insure our taking the sample in the same way, but as now written they do not insure our taking the sample from the same bags or even the same portion of the pile. Perhaps the directions cannot be made sufficiently explicit to cover this, but you have asked for criticisms and in my opinion this is about the only thing that can be criticised in these methods.

D. M. Walsh, U. S. Food and Drug Inspection Station, Baltimore, Md.—
Date: February 20, 1926.

The only comment which we desire to make is that it seems that the analytical work contemplated would be very excessive and that the labor of carrying the large number of containers that would be required for one sample would probably preclude sampling any large number of consignments.

F. C. Blanck, Bureau of Chemistry, Washington, D. C.—
Date: June 10, 1926.

I do not favor changing of the phraseology so as to provide for a composite sample of a flour shipment for a moisture determination. It seems to me that moisture determinations on the individual samples are just as important in the case of flour as individual analyses are in the case of butter shipments. With reference to the actual method of sampling, it seems to me that we must consider two purposes for the sampling of flour—one, what might be termed the commercial sampling, and the other, official sampling for moisture and other chemical determinations. For commercial purposes, the sample or samples obtained should undoubtedly be representative of the entire parcel of flour and so would include all of the entire sample. For official regulatory purposes, however, the moisture content of the center of the sack would seem to be the one on which judgment as to possible legal action would be based. These two viewpoints seem conflicting.

These comments were carefully considered by the committee. It is noted that two points are covered: first, the question of indicating more specifically the sacks that should be sampled; and second, the question of compositing the individual samples drawn from each pile of flour. It did not seem that more specific directions could be drawn for selecting the sacks from the pile because of the various methods of piling flour, there being no standard method. It also did not seem to be advisable to include in the method the compositing of samples for the reason that a more accurate estimation of the shrinkage can be made from the moisture and weight of the individual sacks.

Plans were accordingly made to do some collaborative work. The questions to be solved by experimentation seemed to be divided into three parts: First, whether or not the method will give a true sample from an individual sack after there has been some loss in moisture; second, whether a pile of sacks can be sampled by two individuals so that the samples will check as to moisture content; third, whether the sample drawn from the pile by this method actually represents the moisture content of the pile.

As the third question was regarded as one extremely difficult to answer by experimentation, it seemed desirable to leave it for later decision and to use for present purposes the general agreement reached by the various trade organizations involved. This agreement and the experimental work done by A. E. Paul and G. J. Morton and reported previously[1] seemed to justify the postponement. However, experimentation seemed more advisable in the case of the first two questions, and the following tests, "A" and "B", were accordingly devised:

EXPERIMENT "A".

A. O. A. C. Sampling of Flour.

Sample a 49 pound sack of flour by the tentative method attached. (See also *J. A. O. A. C.*, 1926, **9**: 39.)

Empty the flour into a milk can, ice cream can, or other closed receptacle large enough to contain all the flour when about two-thirds full. Alternately invert and roll the closed can fifty times, and draw five cores from top to bottom of the flour in the can to secure a thoroughly representative sample. Return the flour to the sack and after it is sewed, sample again by the tentative method. Draw these three samples in close order, preventing contact with the air as much as possible, using a one pint Mason jar provided with a rubber gasket as a container.

Determine moisture at once in triplicate by the vacuum method described on page 39, *J. A. O. A. C.*, Vol. 9, No. 1, or forward to U. S. Food and Drug Inspection Station, 310 Federal Bldg., Minneapolis, Minn.

Weigh the sack of flour to at least one-fourth ounce. Allow it to lie on the floor or table in a warm, dry place (approximately 60° to 75°F. and 45–55 per cent humidity—similar to an ordinary store room for flour) for seven days and reweigh on the same scales, taking the necessary precautions to prevent any sifting.

After the reweighing repeat the sampling in the sack, mixing in the can and sampling, the sampling after the flour is resacked, and the analysis or other disposition outlined in the first and second paragraphs above.

Dated: Minneapolis, Minn., March 27, 1926.

EXPERIMENT "B".

A. O. A. C. Sampling of Flour.

Draw two sets of samples by the tentative method attached (see also *J. A. O. A. C.*, 1926, **9**: 39) from the same pile of flour. Select a pile of at least 100 sacks that have been stored for some time (5–15 days in a warm, dry storeroom). Use a one pint Mason jar equipped with a rubber gasket as a container.

Determine moisture at once in triplicate on each subdivision by the vacuum method described on page 39, *J. A. O. A. C.*, Vol. 9, No. 1, or forward to U. S. Food and Drug Inspection Station, 310 Federal Bldg., Minneapolis, Minn.

This experiment is designed to find the accuracy of the sampling method when used on the same pile of flour. Select a different set of sacks for the second set of samples than those from which the first set of samples is drawn. It is suggested that the two samplings be done simultaneously by different men.

Dated: Minneapolis, Minn., March 27, 1926.

The results of these experiments are given in Tables 1 and 2. Grateful acknowledgment is made to F. A. Collatz, Chief Chemist, Washburn Crosby Co., Minneapolis, Minn.; M. A. Gray; A. W. Garrett, Food and Drug Inspector, U. S. Food and Drug Inspection Station, Minneapolis, Minn., and to E. F. Gill, Head Miller, Duluth Universal Milling Company, Duluth, Minn., for their assistance in completing these experiments.

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[1] *This Journal*, 1926, **8**: 680.

Table 1 shows the accuracy obtained by the use of the tentative method when applied to a single sack of flour. The percentage of moisture reported in the first, third, and fourth columns, headed "calculated from weight loss", "flour mixed in closed can", and "re-sacked flour sampled by tentative method", respectively, should check closely. These data represent three separate efforts to arrive at the true moisture content of the sack of flour. The figures in the second column, headed "sack sampled by tentative method", when compared with those in the other three columns show the accuracy of the tentative method when applied to a single sack of flour.

In the experiment reported 10–8–26, the figures do not check. The figure in the last column, "resacked flour sampled by tentative method", checks the calculated moisture. There is a bare possibility that the flour in the closed can was not mixed sufficiently, although the directions were followed carefully. It is highly probable that the moisture content of this sack of flour after the last storage period was very close to 11.88 per cent, as shown by the calculated moisture. Assuming that 11.88 per cent is the correct moisture content of the sack, the difference between the correct moisture and the moisture found when the sack was sampled by the tentative method is 0.38 per cent, which appears to be excessive.

In the experiment dated 10–4–26, the three check determinations given in columns one, three, and four are quite uniform. Assuming that the true moisture content is 12.22 per cent, the variation of the

TABLE 1.

Results of Experiment "A".

(To show the accuracy with which the tentative method draws a representative sample from one sack of flour.)

SAMPLER	DATE	PERCENTAGE OF MOISTURE AVERAGE OF TRIPLICATE DETERMINATIONS			
		Calculated from weight loss	Sack sampled by tentative method	Flour mixed in closed can	Resacked flour sampled by tentative method
Runkel*	9–3–26	....	13.70	13.78	13.65
	9–28–26	12.82	12.83	12.81	12.81
	10–8–26	11.88	12.26	12.16	11.81
Collatz	9–24–26	....	....	13.46	13.48
	10–4–26	12.22	12.60	12.15	12.22

* Analyses were made by M. L. Johnson, U. S. Food and Drug Inspection Station, Minneapolis, Minn.

moisture when sampled by the tentative method is 0.38 per cent, which also seems quite large.

Of this experimental work the tentative method gave checks close to the actual moisture in two instances, but in the other two the difference was 0.38 per cent, the tentative method giving high results in both cases. While the demonstrated accuracy of the tentative method is not regarded

TABLE 2.

Results of Experiment "B".

(To show the accuracy with which one sampler may check another on the same pile of flour by the use of the tentative method.)

LOCATION OF PILE	PERCENTAGE OF MOISTURE AVERAGE OF TRIPLICATE DETERMINATIONS		
	Sack No.	Collatz "A"	Collatz "B"
Washburn Crosby Mill Minneapolis, Minn.	1	13.44	13.50
	2	13.46	13.54
	3	13.48	13.44
	4	13.46	13.52
	5	13.43	13.30
	6	13.46	13.51
	7	13.47	13.40
	8	13.48	13.46
	9	13.40	13.46
	10	13.46	13.46
	Average	13.45	13.46
	Sack No.	Gray "1"	Gray "2"
Pillsbury Mill* Minneapolis, Minn.	1	13.36	13.43
	2	13.40	13.36
	3	13.38	13.53
	4	13.39	13.47
	5	13.32	13.35
	6	13.44	13.38
	7	13.35	13.22
	8	13.38	13.45
	9	13.39	13.37
	10	13.38	13.38
	11	13.38	13.38
	12	13.41	13.46
	Average	13.38	13.40
	Sack No.	Garrett	Gill
Duluth Universal Mill* Duluth, Minn.	1	13.61	13.53
	2	13.56	13.55
	3	14.18	14.03
	4	13.60	13.55
	5	13.58	13.55
	6	13.62	13.53
	7	13.59	13.55
	8	13.65	13.55
	9	13.62	13.66
	10	13.64	13.62
	Average	13.66	13.61

* Analyses were made by M. L. Johnson.

as good, there is a question in the minds of some of the committee whether any other method of sampling will give more accurate results. The quantity of data is rather meager, because the experimental work is rather involved, requires considerable time, and is very susceptible to errors. Further data seem desirable before the method is adopted as official.

It is therefore recommended[1] that collaboration be secured for further work according to Experiment "A", both the tentative method and one other method of drawing cores from the sack being used.

Table 2 shows the agreement with which two samplers checked by the tentative method. It is not believed that this experimentation is sufficient, since the analyses of the various sacks are fairly uniform throughout the pile. It does not appear, therefore, that the piles of flour were of sufficient variation in themselves to give the method thorough trial, although the experimenters endeavored to locate piles of flour which they considered to be difficult to sample. It is recommended that further collaborative work according to Experiment "B" be done during the coming year.

REPORT ON MOISTURE IN FLOUR AND ALIMENTARY PASTES.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The collaborative work of the past year was limited to a study of the so-called routine method for the quantitative estimation of moisture by drying the samples of flour at 130°C. for a period of one hour[2].

The collaborators' samples were prepared March 15 by thoroughly mixing the contents of a 24 pound sack of a patent flour made from a blend of hard and soft wheats. The mixture was then poured into glass fruit jars, which were tightly sealed and forwarded to the collaborators.

Owing to changes in moisture content that seem to be inevitable in any sample of flour, the collaborators were urged to weigh out the flour charges into the moisture dishes as soon as the samples were received, since a strict adherence to this rule would permit of more concordant results and would be more creditable to the collaborators, besides doing greater justice to the method.

Collaborators were asked to observe the following conditions:

Make three determinations the first day and three the second—six in all.

If possible, use aluminum dishes, cylindrical in shape, 18 mm. high and 60 mm. in diameter with close-fitting inside covers.

Have no other samples in the oven at the same time.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 78.

[2] *This Journal*, 1925, 8: 310.

Tentative Method.

Weigh accurately about 2 grams of the sample in a tared, covered dish. Remove the cover and heat the dish and contents in air in an oven at 130°C. for 1 hour. Replace the cover on the dish and cool in a desiccator for 20 minutes. Weigh and calculate the loss in weight as moisture.

The results obtained by 19 collaborators are shown in the table. The collaborators were the following:

(1) Samuel Alfend, Old Custom House, St. Louis, Mo.
(2) L. H. Bailey, Bureau of Chemistry, Washington, D. C.
(3) M. J. Blish, University of Nebraska, Lincoln, Nebr.
(4) Mary M. Brooke, Grennan Bakeries, Inc., Detroit, Mich.
(5) A. Christie, Bureau of Agricultural Economics, Washington, D. C.
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(16) O. C. Racke, American Diamalt Co., Cincinnati, Ohio.
(17) G. A. Shuey, Pennsylvania State College, State College, Pa.
(18) F. T. Shutt, Experimental Farm, Ottawa, Canada.
(19) G. C. Spencer.

Collaborative results on determination of moisture in flour.

COLLABORATOR	DATE	FIRST DAY			SECOND DAY			AVERAGE
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	March 22	13.80	13.84	13.87	13.92	13.87	13.88	13.86
2	March 26	13.83	13.82	13.82	13.86	13.82	13.82	13.82
3	April 3	13.54	13.58	13.57	13.61	13.59	13.54	13.57
4	April 30	13.46	13.44	13.44	13.45	13.46	13.46	13.45
5	March 23	13.79	13.75	13.79	13.82	13.79	13.80	13.78
6	March 24	13.76	13.77	13.75	13.83	13.83	13.78	13.78
7	March 25	13.78	13.74	13.71	13.86	13.84	13.80	13.78
8	March 22	13.79	13.90	13.81	13.84	13.75	13.79	13.81
9	April 5	13.60	13.64	13.59	13.61	13.70	13.57	13.61
10	June 24	13.84	13.79	13.79	13.86	13.88	13.88	13.84
11	March 19	13.72	13.78	13.73				13.74
12	May 15	13.33	13.35	13.38	13.55	13.52	13.50	13.43
13	March 22	13.94	14.02	13.90	13.97	14.06	13.88	13.96
14	March 24	13.71	13.64	13.67	13.74	13.70	13.77	13.70
15	March 22	13.90	13.90	13.89	13.89	13.89	13.86	13.88
16	April 1	13.72	13.72	13.70	13.70		...	13.71
17	May 2	13.10	13.06	13.15	13.05	13.02	13.14	13.08
18	April 26	13.75	13.72	....	13.81	13.79	13.74	13.76
19	March 25	13.62	13.68	13.66	13.67	13.67	13.64	13.65
						Final	average	13.69

DISCUSSION OF RESULTS.

An examination of the table will serve to demonstrate the difficulties that attend the preparation of representative samples for collaborative workers.

Eleven of the results were reported as being obtained during the month of March. The average of these eleven results was 13.79 per cent. The average for the month of April was 13.62 per cent and for May and June, 13.45 per cent. These figures indicate a steady decline in apparent moisture content of the flour from March to May.

The method itself, as applied by the several workers, seems to confirm all previous claims for its reliability. With one exception, all analysts reported a variation in results from the highest to the lowest of 0.15 per cent or less.

RECOMMENDATIONS[1].

It is recommended that the method of drying flour for one hour at 130°C., known as the "tentative" or "substitute" method, be adopted as official, first reading.

REPORT ON ASH IN FLOUR AND GASOLINE COLOR VALUE.

By D. A. Coleman (Bureau of Agricultural Economics, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee.*

The investigations conducted by the associate referee on ash were planned to obtain a comparison of the A. O. A. C. method for ashing flour[2] and the methods used for ashing flour in various laboratories, as well as to answer queries regarding the technique of the ash test. Additional information was likewise sought regarding the use of the temperature 550°C. for ashing all classes and grades of flour.

In order to study the first point, samples of flour representing a short patent, a standard patent, and a first clear spring wheat flour known to be difficult to ash, were sent to various laboratories having temperature control on their ashing furnaces, with a request that they be ashed by the A. O. A. C. method and also by the method used by each laboratory as a routine method.

The results of these tests are given in Table 1. In Columns 2, 3, and 4 are given the ash results as obtained by the methods in use as routine in the collaborators' laboratories, and in Columns 5, 6, and 7 those obtained on the same samples of flour ashed by the A. O. A. C. method.

The ashing temperatures employed by the different collaborators varied from 540° to 650°C. Some collaborators used the same temperature of ashing throughout the test, whereas others varied the temperature in stages, exposing the ash to high heat for a short period at the close of the ashing period.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 78.
[2] *Methods of Analysis, A. O. A. C.*, 1925, 225.

TABLE 1.

Comparison of ash results obtained by A. O. A. C. method and individual laboratory methods.

COLLABORATOR	COLLABORATOR'S METHOD			A. O. A. C. METHOD		
	Short Patent	Standard Patent	First Clear	Short Patent	Standard Patent	First Clear
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.413	0.485	0.773	0.420	0.482	0.780
2	0.420	0.486	0.780	0.429	0.495	0.813
3	0.401	0.467	0.769	0.402	0.468	0.768
4	0.430	0.486	0.784	0.422	0.480	0.786
5	0.430	0.490	0.790	0.430	0.488	0.792
6	0.436	0.490	0.786	0.428	0.496	0.786
7	0.420	0.482	0.782	0.424	0.486	0.784
8	0.430	0.510	0.810	0.430	0.520	0.810
9	0.400	0.505	0.780	0.400	0.530	0.783
10	0.420	0.480	0.780	0.420	0.480	0.790
11	0.416	0.484	0.786	0.414	0.487	0.780
12	0.423	0.493	0.773	0.413	0.480	0.783
Average	0.419	0.488	0.782	0.419	0.491	0.788
Maximum	0.436	0.510	0.810	0.430	0.530	0.813
Minimum	0.400	0.467	0.769	0.400	0.468	0.768
Range	0.036	0.043	0.041	0.030	0.062	0.045

The results obtained by the individual laboratory methods agree with the results obtained by the A. O. A. C. method very closely. Where differences occur the tendency was in the same direction by either method, indicating that some other item than that of method entered into the final results. On the other hand, even though the averages of all results do not bring the fact out clearly, the results obtained by the use of the A. O. A. C. method were slightly higher in many instances than the results obtained by the use of any of the individual laboratory methods.

In submitting their results the majority of the collaborators questioned whether the temperature 550°C. was not too low for ashing these flours, as the appearance of the ash in some instances indicated incomplete incineration even over an extended period of time—in one case 28 hours. If such were the case, the *very slightly* higher A. O. A. C. results might be accounted for.

More definite information regarding the use of 550°C. for ashing various classes of flours was sought, and for this purpose flour milled from five of the commercial classes of wheat grown in various parts of the United States was ashed. The results of these tests will be found in Table 2.

As a result of this study, it was found that 36 out of the 60 samples of flour tested ashed to a fluffy, light grey ash by the A. O. A. C. method.

TABLE 2.

*Effect of temperature upon the percentage, color and condition of ash in various classes of straight grade flour**.

SAMPLE NO.	CLASS OF FLOUR	STATE WHERE WHEAT WAS GROWN	PERCENTAGE OF ASH AT TEMPERATURE INDICATED			
			550°C.	575°C.	600°C.	620°C.
1	Hard Red Spring	Nebraska	0.628†	0.617†	0.585†‡	0.587†‡
2	"	Minnesota	0.647	0.657	0.627	0.633
3	"	Nebraska	0.540	0.537	0.523	0.513
4	"	Washington	0.612†	0.598†	0.590†‡	0.603†‡
5	"	Minnesota	0.572†	0.531†	0.525†‡	0.540†‡
6	"	"	0.627†	0.617†	0.590†‡	0.603†‡
7	"	"	0.550†	0.540†	0.520†‡	0.507†‡
8	"	"	0.650†	0.660†	0.610†‡	0.600†‡
9	"	"	0.480†	0.487†	0.457†‡	0.457†‡
10	"	"	0.560†	0.567†	0.510†‡	0.513†‡
11	"	"	0.610†	0.623†	0.583†‡	0.597†‡
12	"	"	0.547†	0.560†	0.510†‡	0.533†‡
13	"	"	0.613†	0.600†	0.577†‡	0.580†‡
14	"	Montana	0.803	0.797	0.787	0.793‡
15	"	N. Dakota	0.563†	0.577†	0.577†‡	0.557†‡
16	"	Wisconsin	0.550†	0.543†	0.527†‡	0.517†‡
17	Hard Red Winter	Nebraska	0.467	0.493	0.450	0.443
18	"	"	0.432	0.422	0.398	0.393
19	"	"	0.500	0.507	0.480	0.473
20	"	"	0.540	0.560	0.507	0.517
21	"	"	0.547	0.543	0.487	0.493‡
22	"	Washington	0.553	0.527	0.487‡	0.487‡
23	"	"	0.553	0.557	0.533	0.540
24	"	"	0.527†	0.547†	0.507†‡	0.487†‡
25	"	"	0.517	0.513	0.507‡	0.490‡
26	"	Minnesota	0.550	0.499	0.489	0.450‡
27	"	"	0.540	0.520	0.500	0.513‡
28	"	"	0.443	0.450	0.427	0.427‡
29	"	"	0.550	0.567	0.533	0.530‡
30	"	"	0.423	0.437	0.393	0.403‡
31	"	"	0.600	0.603	0.550	0.560‡
32	"	"	0.500†	0.500†	0.477†	0.477†‡
33	"	"	0.557	0.563	0.540	0.530‡
34	"	Wisconsin	0.550†	0.567†	0.543†‡	0.530†‡
35	"	"	0.443†	0.447†	0.435†‡	0.420†‡
36	Soft Red Winter	Minnesota	0.473	0.453	0.440	0.453
37	"	"	0.627	0.637	0.580	0.590‡
38	"	Washington	0.383	0.393	0.347‡	0.360‡
39	"	"	0.490†	0.477†	0.467†‡	0.457†‡
40	"	Pennsylvania	0.480†	0.473†	0.467†‡	0.450†‡
41	"	"	0.590†	0.603†	0.580†‡	0.517†‡
42	White	Washington	0.425	0.400	0.364‡	0.400‡
43	"	"	0.499	0.485	0.475‡	0.488‡
44	"	"	0.470	0.467	0.453	0.440
45	"	"	0.697†	0.700†	0.667†‡	0.650†‡
46	"	"	0.513	0.513	0.467	0.463‡
47	"	"	0.520	0.533	0.477‡	0.490‡
48	"	"	0.473	0.483	0.423‡	0.420‡
49	"	"	0.552	0.490	0.463‡	0.497‡
50	"	Idaho	0.533†	0.543†	0.520†‡	0.517†‡
51	"	"	0.547	0.560	0.503‡	0.510‡
52	"	Montana	0.500	0.493	0.477	0.483‡
53	"	Idaho	0.443	0.447	0.443	0.433
54	"	"	0.480	0.487	0.473	0.460
55	"	"	0.420	0.420	0.433	0.433
56	"	"	0.460	0.470	0.477	0.470
57	"	"	0.427	0.443	0.383‡	0.393‡
58	Durum	Minnesota	0.730	0.691	0.653‡	0.663‡
59	"	"	0.795	0.760	0.712	0.740
60	"	"	0.650	0.623	0.608	0.617

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* The data given in this table were contributed by H. C. Fellows, Grain Division, Bureau of Agricultural Economics.

† Ash not satisfactory in appearance.

‡ Fusion had taken place.

At the temperature recommended in this method, namely, 550°C., the ash from 24 of the samples of flour was unsatisfactory as it was black or very dark grey in color. Increasing the temperature to 575°C. or to 600°C. did not materially change the appearance of the ash in these 24 flours. At 620°C., 9 of the 24 samples gave an ash of a good color. This improvement, however, was accompanied by a change in the percentage of ash in the sample. Likewise the ash from all these samples fused at this temperature.

The flours milled from the hard red spring wheats were the most difficult to ash as 14 out of the 16 samples tested did not ash to an acceptable condition at any of the temperatures tried. The soft red winter wheat flour was likewise found difficult to ash. On the other hand, only 3 of the flours milled from the hard red winter wheats were found difficult to ash. A similar condition was found ashing flours milled from white wheat as only 2 out of the 16 samples tested did not ash satisfactorily.

No great difficulty was found ashing the 3 samples of durum flour.

The spring wheat flours and the soft red winter wheat flours fused the most readily of all the five classes of flour tested. Thirteen of the 16 spring wheat flours and 4 out of the 6 samples of soft red winter wheat flours fused at 600°C. The hard red winter wheat flours were very resistant to fusion as only 5 out of the 19 samples fused at 600°C.

The ash from the 3 samples of durum flour did not fuse even at 620°C.

The effect of high temperatures on the ash results is not so marked as would be expected. At 550°C. the average of all results was 0.542, at 575°C. it was 0.538, at 600°C. it was 0.511, and at 620°C. it was 0.511 per cent. The average difference in percentage of ash heated at 550°C. and 620°C. was 0.031. The average difference in the ash content of the samples heated at 550°C. and 575°C. was 0.004 and between 550°C. and 600°C., the point where fusion starts, it was 0.031 per cent.

It was noted that in the majority of the determinations that gave unsatisfactory results the ash formed a small button at the bottom of the ashing crucible. In an effort to prevent this formation, glycerol alcohol, as recommended by Bailey and Hertwig[1], and 60 mesh alundum were tried. The data will be found in Table 3.

For making these tests, the samples that had been found difficult to ash were selected. Both glycerol alcohol and alundum prevented the ash from collecting into small hard carbonaceous masses, and in both instances it was spread out over the bottom of the crucible. When alundum was used the ash was invariably white in character, and while there was great improvement in the ash when glycerol alcohol was used, it was not so satisfactory in appearance.

A comparison of the percentage of ash found by the A. O. A. C.

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[1] *Cereal Chem.*, 1924, 1: 82.

TABLE 3.

Effect of glycerol alcohol and alundum upon flour ash results.

SAMPLE NO.	ASHED WITH ALUNDUM		ASHED WITH GLYCEROL ALCOHOL		ASHED BY A. O. A. C. 550°C.	
	Ash	Color	Ash	Color	Ash	Color
	per cent		*per cent*		*per cent*	
6	0.610	White	0.607	Gray	0.627	Black
7	0.543	"	0.537	White	0.550	"
8	0.623	"	0.620	"	0.650	"
9	0.483	"	0.490	"	0.480	"
10	0.560	"	0.557	Gray	0.560	"
11	0.603	"	0.603	White	0.610	"
12	0.557	"	0.560	Gray	0.547	"
13	0.587	"	0.567	Dark gray	0.613	"
15	0.563	"	0.553	Gray	0.563	"
24	0.560	"	0.557	Dark gray	0.553	"
45	0.683	"	0.683	Gray	0.697	"
50	0.560	"	0.537	Gray black	0.553	"

method with the results obtained by the same method when either glycerol alcohol or 60 mesh alundum was used for the purpose of spreading the ash out on the bottom of the crucible, shows that even though the ash was black or gray-black, the weight of such carbon is almost negligible. In only two samples was the change in the weight of the ash measurable when the carbon had been removed.

The temperature 550°C., therefore, is sufficiently high to use for all classes of wheat flour providing the ash does not collect in a small area in the bottom of the ashing crucible. If it should, means must be provided to eliminate it, and for this purpose glycerol alcohol or 60 mesh alundum is suggested.

The temperature at which the ashing process should be started is an important factor in securing a light colored ash, as will be seen from the data secured on this subject when four samples of flour were ashed, the muffle being started cold, at 200°C, at 550°C., and at 575°C. The data are given in Table 4. The ash obtained when the muffle was started cold and at a temperature of 200°C. was black in color. The results were high at 550°C. and the ash was gray, but yet of a color that would cause one to wonder whether ashing was complete. Ashing at 575°C. improved the appearance of the ash.

RAPID ASHING METHODS.

Collaborative work was also carried out in an attempt to develop a more rapid method for ashing flour than that now recommended by the association.

Two methods were studied, the glacial acetic acid method and the alundum method.

The instructions given for making these tests were as follows:

TABLE 4.

Ashing by A. O. A. C. method, starting tests at various temperatures.

SAMPLE NO.	TEMPERATURE OF MUFFLE			
	Cold*	200°C.†	550°C.‡	575°C.¶
1	0.458	0.458	0.426	0.425
2	0.510	0.512	0.488	0.486
3	0.822	0.818	0.781	0.787
4	0.362	0.357	0.365	0.358

* Ash very black.
† Ash very dark—unsatisfactory appearance.
‡ Ash medium gray—not entirely satisfactory.
¶ Ash medium gray.

Glacial Acetic Acid Method.

REAGENT.

Acetate solution.—Prepare a solution of calcium acetate by dissolving 1 gram of C. P. calcium acetate in 100 cc. of warm glacial acetic acid. Add 1 cc. of water and filter into a 200 cc. volumetric flask. Wash the filter with glacial acetic acid and finally make up to a volume of 200 cc. with glacial acetic acid.

DETERMINATION.

Weigh into a tared container 5 grams of flour and carefully measure and add 7.5 cc. of the acetate solution. Tightly roll a half-sheet of 11 cm. ashless filter paper around the tip of the stirring rod, stir the mixture into a smooth paste, and insert directly into a muffle furnace maintained at a temperature of 900°C. (1650°F.) or hotter. (The oven at this temperature is orange in color. At the end of 40 minutes burning should be complete.) Remove the dish, desiccate, and weigh the mixture. Make blank tests by evaporating 7.5 cc. to dryness at 100°C. for 40 minutes. Correct for blanks when reporting ash.

Alundum Method.

Weigh into a crucible or other ashing dish 4 grams of alundum (60-mesh). Ignite the crucible and alundum in a muffle and weigh. Weigh into the tared container 2 grams of flour. Prepare a stirring rod by tightly rolling a small triangular piece of ashless filter paper (a quarter of a piece of 11 cm. paper is very satisfactory) and with this rod carefully and thoroughly mix the flour and alundum. Leave the paper rod with the sample and ignite in a muffle at 550°C., taking care that no part of the ash fuses. Take care also to prevent any loss of alundum by mixing and handling. Note the time required to secure a white ash.

The results obtained by the two rapid methods as compared with the standard A. O. A. C. method are given in Table 5.

The great objection to the glacial acetic acid method is, of course, that of fusing the ash; in addition, there seems to be some difficulty in avoiding spattering. Most of the collaborators were entirely unsympathetic with the test, even though almost equally good results were obtained by it on the three samples of flour tested as by the A. O. A. C. method.

On the other hand, favorable comment was voiced regarding the alundum method. Some collaborators secured a satisfactory ash in less than 2 hours.

TABLE 5.

Ash results by A. O. A. C. method as compared with results obtained by glacial acetic acid method and alundum method.

COLLABORATORS	GLACIAL ACETIC ACID METHOD			A. O. A. C. METHOD		
	Short Patent	Standard Patent	First Clear	Short Patent	Standard Patent	First Clear
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.425	0.470	0.785	0.420	0.470	0.780
2	0.424	0.470	0.766	0.402	0.468	0.768
3	0.436	0.496	0.776	0.422	0.480	0.786
4	0.426	0.492	0.778	0.430	0.488	0.792
5	0.433	0.486	0.790	0.428	0.496	0.786
6	0.415	0.490	0.780	0.424	0.486	0.784
7	0.440	0.510	0.810	0.430	0.520	0.810
8	0.430	0.480	0.770	0.420	0.480	0.790
9	0.420	0.490	0.785	0.414	0.487	0.780
10	0.436	0.500	0.786	0.432	0.495	0.800
11	0.427	0.485	0.795	0.422	0.485	0.790
12	0.420	0.482	0.770	0.415	0.473	0.781
13	0.440	0.468	0.676	0.425	0.480	0.800
14	0.452	0.530	0.814	0.442	0.506	0.823
Average	0.430	0.489	0.776	0.424	0.487	0.790
Maximum	0.452	0.530	0.814	0.432	0.520	0.823
Minimum	0.415	0.468	0.676	0.402	0.468	0.768
Range	0.037	0.062	0.138	0.030	0.052	0.065
	ALUNDUM METHOD			A. O. A. C. METHOD		
1	0.440	0.505	0.805	0.402	0.468	0.768
2	0.430	0.490	0.796	0.422	0.480	0.790
3	0.435	0.480	0.790	0.430	0.488	0.792
4	0.445	0.485	0.802	0.428	0.496	0.786
5	0.424	0.478	0.736	0.424	0.486	0.784
6	0.430	0.500	0.800	0.430	0.520	0.810
7	0.395	0.485	0.780	0.400	0.530	0.783
8	0.410	0.480	0.770	0.420	0.480	0.790
9	0.430	0.495	0.785	0.414	0.487	0.780
10	0.420	0.485	0.765	0.442	0.506	0.823
Average	0.426	0.488	0.783	0.421	0.494	0.790
Maximum	0.445	0.505	0.805	0.442	0.530	0.823
Minimum	0.395	0.478	0.736	0.400	0.468	0.768
Range	0.050	0.027	0.069	0.042	0.062	0.055

GASOLINE COLOR STUDIES.

No data are presented this year on this subject. No work was done owing to lack of equipment among collaborators as well as to lack of interest in the test.

SUMMARY AND RECOMMENDATIONS[1].

The temperature of 550°C. recommended for ashing flour is satisfactory, providing the ash does not form a concentrated mass in the bottom of the crucible. Glycerol alcohol or finely divided alundum will prevent this, alundum being the first selection.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 79.

The glacial acetic acid method studied is not satisfactory. Further study of this particular method should be stopped. On the other hand, the acetic acid method sponsored by Weaver[1] at the June meeting of the American Association of Cereal Chemists is brought to the attention of the association.

The alundum method shows promise and should be given more intensive study.

Careful study should also be given to the nature and kind of losses occurring when ash is fused.

REPORT ON GLUTENIN IN FLOUR.

By M. J. BLISH[2] (Agricultural Experiment Station, Lincoln, Nebr.), *Associate Referee.*

The report of last year by the present Associate Referee on Glutenin indicated that the direct method of Blish and Sandstedt[3] appears to offer a reasonably simple method for the accurate estimation of glutenin in wheat flour, assuming that glutenin is a distinct chemical individual that is soluble in alkali, but not soluble in dilute alcohol or salt solutions. It was also reported that by slightly varying certain details of the specified procedure, without changing the fundamental principles of the method, variable results are obtained, although if the original procedure is strictly observed, the results are consistent and agree closely with those obtained by the indirect method of Sharp and Gortner[4], which, in the light of the present knowledge of the flour proteins, must be regarded as reasonably accurate. The 1925 report stated, however, that the influence of certain specified factors upon the results obtained by the Blish and Sandstedt method should be more clearly understood before collaborative work preceding its ultimate adoption as an official method is attempted.

During the past year work has been continuously in progress in an attempt to secure better understanding of certain properties of the flour proteins that are considered to have a definite bearing upon their quantitative estimation. A large portion of the data thus accumulated is either incomplete, inconclusive, or negative, although some definite progress has been made.

Since, as indicated in last year's report, variations in the Blish and Sandstedt procedure gave varying glutenin results, much of this year's work consisted of attempts to ascertain the correct method by analyzing the fractions produced by varying the procedure as previously noted.

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[1] Brendel, G. L., Oxygen-acetate Method of Ash Determination in Flour. *Cereal Chem.*, 1926 3: 222.

[2] Presented by F. C. Blanck.

[3] *Cereal Chem.*, 1925, 2: 57.

[4] Minnesota Tech. Bull. 19, 1923.

These fractions were hydrolyzed, and the products of hydrolysis were estimated by Van Slyke's procedure[1]. The results have thus far been unsatisfactory and inconclusive. The Van Slyke method, although the best available, is not sensitive and accurate enough to detect the extent to which a given protein may be contaminated with small quantities of another protein. The limitations of the Van Slyke procedure may be further emphasized by noting the very appreciably different results which have been obtained from its use by different workers with the same protein. The inevitable conclusion is that there is as yet no method for estimating the products of protein hydrolysis which is of sufficient precision for use in judging the accuracy of procedures for the quantitative separation of individual proteins (except within very wide limits).

Present knowledge of the various physical and physico-chemical properties of proteins (especially with regard to their precise measurement and the factors affecting them) is still too limited to permit of its useful application to the problem at hand.

Since slight contaminations of one flour protein by another cannot be accurately detected by any physical or chemical means now available, reliance can only be placed on Osborne's[2] characterization of the wheat proteins, based upon their solubilities in various solvents. For quantitative purposes, the precautions necessary when using these various solvents in order to reduce errors arising from certain "overlapping solubilities", have been stressed in previous reports. These precautions are observed in Sharp and Gortner's indirect glutenin method, mentioned previously, and results obtained by this method, or by any method giving similar values, must be regarded as the most accurate that can be obtained for glutenin at the present time.

During the past year additional evidence has been secured which shows that the direct glutenin method of Blish and Sandstedt gives results that are in complete agreement with those obtained by the indirect method of Sharp and Gortner. After trying both methods with thirty or more flours at the University of Minnesota, Gortner[3] states that only two showed differences which one could regard as larger than experimental error.

In addition to this evidence two other procedures were devised and tried in this laboratory, both of which gave glutenin results that agree closely with those obtained by the method of Sharp and Gortner. These two procedures are, briefly, as follows:

METHOD 1.

This method is based upon a chance observation that when two or three volumes of pure methyl alcohol are added to one volume of a solution of glutenin in barium hydroxide, the glutenin is precipitated. Gliadin does not precipitate under similar conditions. In applying this principle to flour, 0.5 gram of solid barium hydroxide was added to 8 grams of flour in a 200 cc. flask; the mixture was thoroughly digested with 50 cc. of water, and shaken at frequent intervals for 1 hour. Methyl alcohol was then added to the mark, the flask was thoroughly shaken several times, and the material was allowed to settle as completely as possible. The protein determined in an aliquot of the supernatant extract, subtracted from the total flour protein, gives glutenin. This procedure was tried with seven flours, and in every case the results agreed closely with those obtained by the Blish and Sandstedt method on the same flours.

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[1] *J. Biol. Chem.*, 1911, **10**: 15-55.

[2] The Proteins of the Wheat Kernel. Carnegie Institution, 1907.

[3] Personal communication.

METHOD 2.

The second method was designed to give a direct determination of glutenin at a low concentration of hydroxyl ions, and, if possible, in the entire absence of neutral salts, aside from those in the flour itself. Eight grams of flour in a 200 cc. flask was digested (preferably in a shaking machine) at room temperature with 50 cc. of approximately 0.5 *N* ammonia solution, for several hours, glutenin and gliadin being soluble in this reagent. The flask was then filled to the mark with methyl alcohol and shaken vigorously, and the starch was allowed to settle. A 50 cc. aliquot of the extract was then introduced into a 100 cc. centrifuge tube, placed in a water bath at 50°C., and aerated vigorously with carbon dioxide-free air, amyl alcohol or other suitable foam-preventive being used. The ammonia, as well as the alcohol, was entirely removed by this treatment in about 2 hours, and the suspension became neutral to brom-thymol blue. When made up to approximately its original volume with methyl alcohol and shaken vigorously, the gliadin is redissolved and the glutenin settles rapidly to the bottom. The tube was then whirled in the centrifuge, the supernatant gliadin solution was poured off, and the glutenin was transferred to a Kjeldahl flask and subjected to the usual nitrogen determination. Although this procedure was used on but two flours, it gave results agreeing closely with the other three methods, and it appears to be sound in principle. However, it is somewhat time-consuming and is not to be recommended for routine procedure.

Thus it seems that there are at least four distinct methods for determining glutenin in wheat flour, two direct and two indirect. The fact that all these methods give essentially the same results appears to the writer to be the best obtainable evidence that glutenin values obtained by any one of them must be considered as reasonably accurate. It must be noted that the small quantities of albumin and globulin that are believed to be constituents of wheat flour are not definitely accounted for in any method except that of Sharp and Gortner, although Blish and

Sandstedt secured evidence that these constituents remain in solution in their procedure.

Logically, the next order of procedure will be to select one of the four methods as a tentative method, and since all yield the same results, the matter is reduced to the selection of the simplest and easiest one of the four. From the latter viewpoint, the choice lies between the method of Blish and Sandstedt and the barium hydroxide method (Method 1 in this report). These two methods should be submitted to collaborative studies during the coming year, with a view to selecting as a tentative method the one that gives the more uniform results.

In addition to the observations just reported, this laboratory has recently investigated another point which is of interest in connection with the quantitative estimation of wheat-flour proteins. Bailey and Blish[1] observed that hot alcohol extracts considerably more flour protein than does cold alcohol. Obviously, if hot instead of cold alcohol is used in the method of Sharp and Gortner, a much larger amount of protein is extracted, and considerably lower glutenin values are the result. Recent unpublished experiments by the writer have shown that even after flour has been repeatedly extracted with 70 per cent alcohol at room temperature, until no more protein can be removed by this means, a very considerable amount is further extracted if the flour is treated with boiling 70 per cent alcohol under a reflux condenser. Without giving details, which will be published later, it may be said that this protein material is, in all probability, a product resulting from the splitting off of a certain portion of the glutenin molecule by the hot alcohol treatment. This view is based on a further observation that when purified glutenin was subjected to treatment with boiling alcohol, a considerable portion of it was dispersed, and its Van Slyke numbers were found to vary considerably from those of either the original glutenin or gliadin or the residue remaining after the hot alcohol treatment. This indicates that hot alcohol treatment is not permissible if Osborne's generally accepted ideas of the respective individualities of the wheat flour proteins are to be retained.

SUMMARY.

1. Accepting Osborne's ideas regarding the nature and identity of wheat-flour glutenin, the indirect method for its quantitative estimation proposed by Sharp and Gortner must be regarded as reasonably accurate.

2. Three other methods giving results in close agreement with that of Sharp and Gortner are available. Two of these are superior to the method of Sharp and Gortner on account of simpler and shorter technique. These are the method of Blish and Sandstedt, and the "barium hydroxide" method (Method 1).

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[1] *J. Biol. Chem.*, 1915, 23: 345.

3. It is recommended[1] that these two methods be submitted to collaborative trials, with a view to the selection of one of them as a tentative method.

4. Boiling 70 per cent alcohol removes from wheat flour, in addition to gliadin, some protein material which is called glutenin, accepting Osborne's definition of this protein. However, this protein material removed by hot alcohol differs in chemical composition from either glutenin or gliadin, and there is good evidence that a certain portion of the glutenin molecule is split off by boiling alcohol. Therefore, in the quantitative estimation of the wheat-flour proteins, the use of hot alcohol is not permissible under the present conceptions of the number and respective identities of the proteins of wheat flour.

REPORT ON THE HYDROGEN-ION CONCENTRATION OF FLOUR.

By C. H. BAILEY[2] (Agricultural Experiment Station, St. Paul, Minn.), *Associate Referee.*

The associate referee recommended last year that in the collaborative studies of 1926 the newly appointed associate referee (a) prescribe a method based upon the methods now in use, (b) distribute a buffered solution to the collaborators, (c) urge collaborators to complete their determinations within a period of 5 days, and (d) subject the quinhydrone and other electrodes to collaborative studies as soon as this can be arranged.

A survey of the methods employed by many of the laboratories engaged in hydrogen-ion determinations indicated that in the majority of cases a ratio of 1 gram of flour to each 10 cc. of water was used in the preparation of the flour extract. This was evident from the data included in the report of the associate referee in 1925, as well as in the report of Weaver[3] to the American Association of Cereal Chemists. The associate referee conferred with Weaver, and it was agreed that this ratio of flour to water, 1 : 10, would be recommended to the American Association of Cereal Chemists, and that it would also be employed in the A. O. A. C. collaborative studies.

Certain objections to this detail of the procedure might be raised. Thus, it might be argued that the ratio of flour to water in the preparation should approach as closely as possible that which is encountered in a dough. The associate referee varied the ratio of flour to water through a wide range at one time, however, and found little variation in the hydrogen-ion concentration of the resulting extracts. It accordingly appeared that the ratio of 1 : 10 would give results similar to those obtained through the use of more concentrated preparations and would, moreover, be much more convenient to handle.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 79.
[2] Presented by F. C. Blanck.
[3] *Cereal Chem.*, 1926, **3**: 281.

A temperature of 25°C. was specified in outlining the extraction procedure because this temperature can be conveniently maintained at different seasons in the average laboratory. A 30 minute extraction period, with continuous or intermittent shaking such as will keep the flour particles in suspension, followed by a period of 10 minutes when the preparation should stand undisturbed, was prescribed. Previous investigations had indicated that such treatment will yield an extraction of the same degree of acidity as is obtained by longer extraction. The advantages of as short a period as is feasible will be obvious.

COLLABORATIVE WORK.

Two samples of hard spring wheat flour, (1) a 75 per cent extraction patent and (2) a 25 per cent extraction clear grade, were secured from the Minnesota State Experimental Flour Mill. Each sample was thoroughly mixed, and 40 gram portions were placed in clean rubber pouches. Pouches of different colors were employed for the different samples, and the color of the pouch could thus be used as a means of identifying the samples. Collaborators accordingly reported their results under the designation of the color of the rubber pouch in which the samples were received.

Collaborators were also requested to determine the hydrogen-ion concentration of the flour samples during a period of 5 days between August 31st and September 4th, 1926, inclusive. It is believed that this was done by all the collaborators except one, who made the determination a few days later. Since the samples were secured and distributed the previous week it follows that the time of storage was short and fairly uniform. This obviated variations in acidity of the different samples, which might result from variations in the length of the storage period.

The details of the method which the collaborators were asked to use were essentially as follows:

METHOD.

Weigh 10 grams of flour into a clean, dry 250 cc. Erlenmeyer flask and add 100 cc. of distilled water at 25°C. Shake until the flour particles are in a uniform suspension free from lumps. Either shake continuously, or intermittently, so as to keep the flour particles in suspension for 30 minutes, maintaining at 25°C. during this period. Then discontinue shaking and allow to stand quietly for 10 minutes. Decant the supernatant liquid into the hydrogen electrode vessel and at once determine its hydrogen-ion concentration electrometrically.

Collaborators were also supplied with an aliquot of a Palitzsch buffer solution. This solution, containing sodium chloride, boric acid, and borax, was prepared with a ratio of the last two ingredients which should result in a hydrogen-ion concentration of pH = 8.49. The Palitzsch solution was selected for this purpose because it is so heavily buffered as to be affected very little by the glass of the container, and it remains sterile indefinitely.

Several types of hydrogen electrode vessels were used by the collaborators, the Hildebrand (bubbling) and the Bailey (hand-shaken) vessels being most common. One collaborator used a special bubbling type electrode, and a quinhydrone electrode as well. The concentration of potassium chloride in the calomel electrode vessel was 1.0 *N* in half the instances, and saturated in the other half.

No opportunity was afforded the associate referee for a comprehensive study of the adaptation of the quinhydrone electrode to the examination of flour. One collaborator (No. 2) reported results with such an electrode which agreed with results of the determination with ordinary hydrogen-electrode vessels. Denham and Blair[1] reported that in their experience the quinhydrone electrode was better adapted for use with flour than either the hydroquinhydrone (hydroquinone and quinhydrone), quinquinhydrone (quinone and quinhydrone), or the quinhydro-quinhydrone (quinhydrone, quinone and hydroquinone) electrodes, as well as the ordinary hydrogen electrode.

A range in hydrogen-ion concentration of the Palitzsch buffer solution from pH = 8.28 to pH = 8.52 was reported by the several collaborators. This is nearly as wide a range as was reported in the instance of the flours. In nine instances the results were within 0.04 of the mean of these nine determinations. The peculiar feature of this situation is the lack of correlation between the deviation from the mean of the pH of the buffer solution and the deviation from the mean of the flour samples. Thus the mean pH of the buffer solution was 8.44, and of the patent flour 6.12. Collaborator No. 12 reported for the buffer solution pH = 8.37, which is 0.07 less than the mean, while for the patent flour he reported pH = 6.34, which is 0.22 more than the mean. On the other hand, Collaborator No. 7 reported a value equivalent to pH = 0.06 more than the mean of the buffer solution, but pH = 0.05 less than the mean of the patent flour. If the collaborators had been advised that the buffer solution should be regarded as having the theoretical pH = 8.49, and had then corrected their findings with the patent flour accordingly, it would have brought the values for the latter closer to the mean in the instances of Collaborators Nos. 1, 2 (bubbling hydrogen-electrode), 3, and 9, but would have increased the deviation from the mean in the instance of Collaborators Nos. 7, 11, and 12. It accordingly appears that while a buffer solution may be useful in checking the electrometric hydrogen-ion set-up, it is not safe to apply corrections when the buffer solution does not yield theoretical values.

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[1] *Cereal Chem.*, 1926, 3: 158.

The results in working with the clear flour were somewhat more uniform than were the results with patent flour. Thus the average deviation from the mean in the instance of the former was pH = 0.05, and of the latter 0.08. This might be anticipated in view of the greater buffer value of the clear grade flour.

There is no evidence, in consequence of these studies, to indicate that there is anything inherently wrong with the method that was outlined for the preparation of the extract used in the determination of the hydrogen-ion concentration of flour. The fact that the collaborators reported results with the buffer solution varying as widely as with the clear grade flour, indicates that the variations in the flour may be attributed largely to actual errors in the electrometric measurements rather than to differences in the properties of the extracts. The associate referee suggests that in the next series of collaborative studies, the hydrogen-ion concentration of the buffer solution be reported to the collaborators, and that they be urged to attempt no measurements with the flour samples until they secure the theoretical pH with the buffer solution.

TABLE 1.

Results of the determination of the hydrogen-ion concentration of two flour samples and of a Palitzsch buffer solution.

COLLABORATOR NO.	TYPE OF H-ELECTRODE	CONCENTRATION OF KCl IN CALOMEL ELECTRODE	PALITZSCH BUFFER SOLUTION*	PATENT FLOUR	CLEAR FLOUR
1	Bailey	1.0 *N*	8.33	5.97	6.05
2	(Bubbling) Quinhydrone	Saturated	8.44 8.49	6.00 6.09	6.34 6.25
3	Bunker (modified)	Saturated	8.39	5.99	6.19
4	Bailey	1.0 *N*	8.51	6.18	6.25
5	Hildebrand	Saturated	8.44	6.14	6.24
6	Bailey	1.0 *N*	8.49	6.15	6.24
7	Bailey	1.0 *N*	8.50	6.09	6.17
8	Bailey	1.0 *N*	8.52	6.17	6.30
9	Hildebrand	Saturated	8.28	6.04	6.25
10	Bailey	1.0 *N*	8.51	6.17	6.20
11	Bailey	Saturated	8.45	6.20	6.19
12	Hildebrand	Saturated	8.37	6.34	6.31

* The buffer solution formula should result in a solution with pH = 8.49.

RECOMMENDATIONS[1].

In view of the conclusion recorded in the preceding paragraph, the associate referee recommends—

(1) That the method presented in this report be approved as a tentative method for the determination of the hydrogen-ion concentration of flour. This method has been published[2].

(2) That collaborative studies be continued during the next year, in which the collaborators be provided with standard buffer solution, to which their set-up shall be standardized before beginning the examination of flour samples.

(3) That the associate referee give attention to the possible use of the quinhydrone electrode in this connection.

No report on gluten in flour was given by the associate referee.

No report on the diastatic value of flour was given by the associate referee.

REPORT ON STARCH.

By O. S. Rask (Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.), *Associate Referee.*

The work on starch this year consisted of a collaborative study of the method recently proposed by the associate referee[3]. The collaborative material was a sample of a commercial brand of gluten flour found on the market in 3 pound packages, the labels of which declared a starch content of 39.6 per cent as determined by the diastase method and a protein content of 44.91 per cent. Fred Hillig of the Bureau of Chemistry found the starch content to be 38.5 per cent as determined by the Hartmann-Hillig modification of the diastase method[4]. Subdivisions of the sample and transcripts of the method were sent out to the several collaborators with instructions that they determine starch in this collaborative material by the method submitted.

A short time after these instructions had been sent out, H. R. Smith, one of the collaborators, suggested that the preliminary extractions with ether, dilute alcohol, and water might be dispensed with in the case of gluten flour. In order to prevent a lumping or caking of the material by a direct contact of the dispersing acid, Smith further suggested that the material be first suspended in 15 to 20 ml. of ice-cold water, to which is then added an equal volume of ice-cold concentrated hydrochloric acid, the dispersing acid as specified in the method being composed of approximately equal volumes of water and concentrated hydrochloric acid. The method was then rewritten, and this modified form was sent out to the collaborators with the additional instructions that they determine starch in the collaborative material by the method so modified as well as by the method in its original form. The method with Smith's suggestions incorporated, reads as follows:

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 79.
[2] *This Journal*, 1927, **10**: 33.
[3] *Ibid.*, 108.
[4] *Ibid.*, 1926, **9**: 482.

Transfer 2 grams of the gluten flour directly to the 100 ml. volumetric flask. Add 15 or 20 ml. of ice-cold distilled water and shake until a uniform suspension results. Then add an equal volume of ice-cold concentrated hydrochloric acid and immediately shake until the starch appears to be gelatinized. Fill the flask to the mark with the hydrochloric acid solution (20.5 to 21.0 grams of hydrochloric acid per 100 ml.) at a temperature not to exceed 18°C., and mix the contents thoroughly by shaking. From this point proceed according to the original instructions.

The collaborative results are given in Table 1.

The concordance of the results obtained by the several collaborators can no doubt be rated as good. Deviations from the averages may be due in part to a lack of familiarity with the method, the technique of which was quite new. If these same collaborators should repeat their services, better or more concordant results would be quite probable.

Further application of the method, especially to products of varied composition, will no doubt reveal ways of improving it. The work so far suggests certain points for further experimental consideration. One pertains to the advisability or necessity of the preliminary washings, a question first raised by Smith. Another pertains to the manner of drying or rendering moisture-free the coagulated starch.

The main reason for considering the elimination of the preliminary washings specified originally is that the method will be shortened accordingly. There is very little reason, however, for believing that better results can be obtained if they are eliminated. On the contrary, starches coagulated from products not previously washed or extracted in this manner may contain larger amounts of impurities. The examined starches coagulated from the gluten flour, i. e., the collaborative material without the preliminary washings, contained 0.9 to 1.1 per cent of protein ($N \times 5.7$), whereas the starches coagulated from the same material, but previously extracted with ether, dilute alcohol, and water, contained 0.1 to 0.3 per cent protein ($N \times 5.7$). The difference between these percentages, i. e., 0.8 per cent, would just about account for the differences between the grand averages obtained with and without the preliminary washings as recorded in Table 1.

The elimination of the preliminary extractions appears to be more

Table 1.

Collaborative results on gluten flour.

(Starch in this material as determined by the diastase method—38.5 per cent*.)

COLLABORATOR	WITH PRELIMINARY WASHING†		WITHOUT PRELIMINARY WASHING‡	
	per cent	*average per cent*	*per cent*	*average per cent*
L. H. Bailey Bureau of Chemistry Washington, D. C.	39.83 39.95	39.89		
J. C. Palmer Bureau of Chemistry Washington, D. C.			40.89 41.15	41.02
M. J. Blish University of Nebraska Lincoln, Nebr.	40.66 40.94 40.50	40.70	40.30 40.80	40.55
C. E. Mangels Agricultural College, Fargo, N. D.	30.96 40.10	40.03		
A. H. Johnson Montana Agricultural Experiment Station, Bozeman, Mont.		40.58		
R. A. Barackman Minnesota State Testing Mill, Minneapolis, Minn.			39.94 39.62	39.78
R. S. Roe Bureau of Chemistry Chicago, Ill.	42.24	42.24	43.74 42.08 42.98	42.93
H. D. Kruse School of Hygiene and Public Health Johns Hopkins University, Baltimore, Md.	41.02 41.24 41.89	41.38	42.72 43.05	42.88
C. E. Goodrich Bureau of Chemistry Washington, D. C.	41.16 40.68	40.92	39.50 39.95 40.64 41.30	40.35
H. R. Smith Bureau of Chemistry Baltimore, Md.			42.22 42.46 42.26 42.77 42.56	42.45
O. S. Rask	41.50 41.68 41.81	41.66	41.93 41.82 41.82 42.00	41.89
Average		40.92		41.48
Maximum deviation from average				
(above)		1.32		1.45
(below)		1.03		1.70

* Determined by Fred Hillig.

† Suggested by H. R. Smith.

‡ Specified in original directions.

detrimental to the technique itself than to the final results. Most collaborators found, when the preliminary extractions had been eliminated, that both the acid dispersion of the starch and also the supernatant acid alcohol solution out of which the starch had been coagulated filtered more slowly, and that more time was required for washing and transferring the coagulated starch to the tared Gooch crucible. One collaborator declared these filtrations to be impossible unless the sample had previously been extracted with ether, dilute alcohol, and water. Other collaborators found it necessary to centrifuge before each of these filtrations when the sample had not previously been subjected to these preliminary extractions. Apparently, therefore, the use of ether, dilute alcohol, or water (or possibly all three of these solvents) removes certain interfering constituents, and thereby facilitates subsequent operations.

The increased or higher resistance to filtration of the acid dispersion of the previously unextracted sample seems to be a matter of viscosity and may be due to electrolytes, which are known to affect appreciably the properties of colloids, especially the viscosities of colloidal dispersions in acids or alkalies. In the case of starch it is also possible that electrolytes depress in some way or other the dispersing action of the hydrochloric acid.

The increased difficulties in filtering the supernatant acid alcohol solution and in washing and transferring the coagulated starch to the crucible are due to a failure of the coagulated starch to settle out of this solution as completely when the preliminary extractions are excluded as it does when the extractions are included. This failure of the starch to settle out prevents such a clean-cut decantation of the supernatant solution of alcohol and acid as is essential to a reasonably rapid filtration of the supernatant solution and a transfer of the starch to the crucible.

From other experimental data not included in this report, the writer has observed that all these difficulties resulting from the elimination of the preliminary extractions vary rather directly with the amount of extractive material known to be present in the original samples. In the case of products that contain relatively little fat, mineral matter, and water-soluble carbohydrates, e. g., a short patent flour, the elimination of the preliminary extractions has only a slight effect on subsequent operations. But products like mill feeds (bran and shorts), which contain relatively large amounts of fat, mineral matter, water-soluble carbohydrates, and also water-soluble proteins, will practically not yield to the method without the preliminary extractions. In the case of unextracted shorts it has been found that 30 to 60 minutes is required for filtering the necessary 50 ml. aliquot of the acid dispersion, even though large quantities of fluffy asbestos are used, and that 60 to 100 minutes more is required for filtering the supernatant acid alcohol solution and transferring the coagulated starch to the crucible. When this same feed is first subjected to the preliminary extractions, these two periods are reduced to not more than 5 minutes each. Apparently, therefore, the elimination of the preliminary extractions can be considered only when less accurate results suffice and when the sample contains very little extractive material. In order that the method may give the most accurate results possible and be applicable to cereal products in general, it will probably be necessary to retain the preliminary extractions, as originally specified.

The coagulated starch is extremely hygroscopic—more so than had been anticipated. It is therefore a separate problem to render and maintain it moisture-free in order that it may be weighed as starch. For expelling moisture, the method in its present form specifies three alternatives. These are as follows:

1. Dry at 105°C., under atmospheric pressure, to a constant weight.
2. Dry at 100°C., in vacuo, to a constant weight.
3. Dry at 130°C., under atmospheric pressure for 1 hour.

None of these procedures is entirely satisfactory. The first and the second will yield satisfactory results in the hands of intelligent and careful workers, but the first consumes much time, and the second requires expensive equipment. The third procedure is free from the objections of the first and the second, but it frequently fails to bring the starch to a reasonably constant weight. However, it is rather probable that this objection can be overcome by prolonging slightly the time of drying. Experience in this laboratory indicates that it will be sufficient to dry for 1 hour and 30 minutes at 130°C. Such a procedure for rendering the starch moisture-free might be considered in a further study of this method.

The coagulated and moisture-free starch absorbs moisture with great avidity. In order, therefore, to keep it moisture-free until it has been weighed it is necessary to observe strictly such precautions as (1) using covers for the crucibles, (2) using good desiccators equipped with the best desiccating agents, and (3) weighing after a definitely specified time, probably 20 or 30 minutes.

Concentration and temperature of the dispersing acid have been specified arbitrarily to a certain extent. A lower concentration with a higher temperature, or vice versa, would no doubt have been equally satisfactory, at least within certain limits. Concentration was standardized against a temperature of 20°–22°C., because this temperature approximates rather closely that of the laboratory, and also that at which volumetric glassware is calibrated. At this temperature a concentration of 19 grams of hydrogen chloride per 100 ml. solution was found to be just about sufficient for dispersing the starches that were examined. In order to provide a factor of safety for more resistant starches, a concentration of 20.5–21.0 grams of hydrogen chloride per 100 ml. solution was specified. It will be remembered that starches dispersed in an acid containing 21 grams of hydrogen chloride per 100 ml. at 25°C. suffered no noticeable hydrolysis during the first 45 minutes. In order to provide a safety factor against hydrolysis of the less resistant starches, a maximal temperature of 22°C. and a maximal time of dispersion of 35 minutes were specified. These specifications, therefore, provide a safety factor of 1.5 grams of hydrogen chloride per 100 ml. against an incomplete dispersion and a safety factor of 10 minutes' time and 3°C. temperature against hydrolysis. It would probably be futile to try to determine any of these values with great exactness because starches vary considerably in their resistances to acid dispersion; nevertheless, a further study of the method might include a reconsideration of these two important points.

Questions have been raised regarding the significance of possible variabilities in the dilution of the dispersing acid on account of corresponding variabilities in the quantities of residual wash water remaining in the sample when the dispersing acid is applied. A little consideration will show that the largest possible variations in this dilution are insignificant. It will be remembered that allowance has previously been made for 1.0 ml. of residual wash water, since the concentration of the dispersing acid has been standardized on the basis of a subsequent 1 per cent dilution by residual wash water. Under no probable conditions would more than 3 ml. of residual wash water be present in the material when the dispersing acid is applied. Under such extreme conditions the contents of the 100 ml. flask would be approximately as follows when made up to the mark:

Residual wash water allowed for	1.00 ml.
Excess wash water	2.00
Sample	1.00
Dispersing acid	96.00

If the dispersing acid contained the maximum specified concentration of hydrogen chloride, viz., 21.0 grams per 100 ml. solution, then under the above conditions the actual concentrations of the dispersing acid as a result of its dilution by the extra 2 ml. of residual wash water would be $\frac{96}{98}$ of 21.0 or 20.58 grams of hydrochloric acid per 100 ml. solution, which is still within the limits specified for the dispersing acid. Accordingly, the dispersing acid undergoes only an insignificant dilution in any probable variation in the amount of residual wash water remaining in the sample when the dispersing acid is applied.

In applying this method, in routine work, to a large number of samples, considerable time could be saved by performing the preliminary extractions simultaneously on several samples, possibly as many as a dozen. However, it is obvious that each sample must be manipulated individually from the point at which the dispersing acid is applied until the crucible containing the coagulated and washed starch is ready for the drying oven. Accordingly, the washed samples that follow other samples in these individual treatments must wait until the work on the others has been completed. Therefore, it is evident that this scheme requires inhibition of diastatic action in those samples that do not receive immediate application of the dispersing acid. Such inhibition might be supplied effectively and conveniently in the following manner: Transfer the washed sample, together with filter paper, to the 50 ml. beaker. Tamp the material with the stirring rod flattened on the end until it is spread as a uniform mat over the entire bottom of the beaker. Then place the beaker on a steam bath until the starch has gelatinized and until the residual washed water has been driven off.

Such a procedure would either destroy any diastase that might be present in the washed sample or render it inactive by a removal of water. In this condition the material could be set aside indefinitely, and the determination resumed and completed at any convenient time. In case the material has been brought to a state of complete dryness on the steam bath it may be necessary to work it up with 1 ml. of water before applying the dispersing acid.

After the method had been sent out to the collaborators some consideration was given to the possibility of coagulating the starch out of the 50 ml. of acid dispersion by a volume of alcohol smaller than that specified, viz. 110–115 ml. It was found that 60 ml. of alcohol was sufficient for producing a complete coagulation. But whenever this volume of alcohol or any other volume less than 100 ml. had been used for coagulation, it was observed that some of the starch would invariably pass through the Gooch asbestos filter in the process of washing with the 70 per cent alcohol. It seems doubtful, therefore, whether a satisfactory coagulation can be produced with less than 110 ml. of alcohol.

The only troublesome step in this method is the slow filtration that occasionally results from a clogging of the Gooch crucible by coagulated starch that is transferred to the crucible more or less unavoidably in decanting the supernatant acid alcohol solution. Recently H. R. Smith suggested that this source of trouble might be eliminated by covering the asbestos pad in the crucible by a layer of alundum or sand grains, 1 to 2 cm. deep. Whenever a clogging layer of starch forms on the sand or alundum, such a layer may be broken up by means of a stirring rod without disturbing the asbestos pad underneath.

The writer takes the liberty at this point to correct an erroneous statement which occurs in Line 2, Note (4)[1] appended to the method previously published. This statement reads as follows: "Lower temperatures are not objectionable". It should read: "Lower temperatures are not objectionable provided the acid dispersion reaches a temperature of 20°C. before it is filtered".[1]

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[1] *This Journal*, 1927 **10:** 114.

REPORT ON CHLORINE IN BLEACHED FLOUR.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

Owing to the late appointment of the present associate referee, little was accomplished on chlorine estimations in flour.

The time available was spent in reviewing the literature on the subject and repeating the method proposed by Armin Seidenberg, the former associate referee[2].

The brief experience thus acquired with the Seidenberg method indicates that the extraction procedure here laid down is satisfactory and that the real problem that is now awaiting solution is the estimation of the chlorine in the extract thus obtained.

RECOMMENDATION[3].

It is recommended that the work on the estimation of flour-bleaching chemicals be continued.

REPORT ON EXPERIMENTAL BAKING TESTS.

By M. J. BLISH[4] (Agricultural Experiment Station, Lincoln, Nebr.), *Associate Referee.*

In February, 1926, the writer was advised by the secretary of this association that he had been designated as Associate Referee on Experimental Baking Tests. In June, 1926, he was appointed chairman of the Committee on the Standardization of the Experimental Baking Test for the American Association of Cereal Chemists. He is therefore serving in essentially the same capacity for two organizations, both working toward a common end, in so far as cereal foods are concerned.

To anyone who makes even the most perfunctory survey of the situation, it is perfectly evident that any useful or satisfactory standardization of the experimental baking test will be a matter of extreme difficulty. The strict standardization that is gradually being accomplished in connection with most of the methods of agricultural chemistry is hardly possible, at the present time, in the case of experimental baking. Nevertheless, there is a persistent and growing conviction among cereal chemists that a serious effort in this direction should be made, and that some phases of the present unsatisfactory situation are susceptible to decided improvement, providing some sort of standardization can be agreed upon.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 80.
[2] *This Journal*, 1925, **8**: 676.
[3] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 80.
[4] Presented by F. C. Blanck.

In studying the situation, especially by means of discussions with prominent cereal chemists, one is first of all impressed with the decided lack of general agreement with regard to even the most fundamental considerations. For instance, there are differences of opinion on the question of the exact nature and scope of the information which an experimental baking test should be expected to reveal. Many are of the decided opinion that since cereal chemists disagree widely as to their respective purposes for conducting experimental baking tests, it is useless to attempt to devise a standard procedure. However, there is a wide-spread feeling that experimental baking can be standardized in certain of its aspects, and the problem then becomes one of restricting any efforts to the particular phase or phases that will meet the requirements of the greatest number of those who are engaged in flour testing from the standpoint of bread-making value, realizing that no single standard test can be devised which will meet all requirements on all occasions.

Preliminary work has been confined necessarily to an attempt to establish a sound and practicable *working basis*, without which no satisfactory or acceptable procedure is likely to be developed. The ideas and opinions of nearly all active and up-to-date cereal chemists have quite recently been canvassed by means of sending a letter and questionnaire to each member of the American Association of Cereal Chemists. Nearly 100 replies to this questionnaire have been received, and among these are the opinions of the most prominent and reputable mill, bakery, industrial, commercial, consulting, and research wheat and flour chemists. More responses will undoubtedly be received. The replies are so varied and numerous that no detailed account of them can appropriately be submitted in this report. The only point of unanimous agreement is that the experimental baking test is the most important flour test. Many express the opinion that it can never be satisfactorily standardized, but the majority feel that some useful standardization can and by all means should be effected.

From the replies received and from other methods of inquiry into the situation, the writer has formulated a tentative outline of procedure, which has recently been approved by the members of the Baking Committee of the American Association of Cereal Chemists. This outline attempts to define the scope of future efforts and to indicate the points upon which emphasis must be placed. The essential part of this outline, which is submitted for approval, is as follows:

THE STANDARDIZATION OF THE EXPERIMENTAL BAKING TEST[1].

Some plausible reasons for the lack of agreement among wheat and flour specialists as to the exact information which a standard experimental baking test should be expected to reveal are as follows:

1. Different purposes for making the test, involving different judging and scoring systems.
2. Different methods of baking.
3. Differences in interpretation of results, either through lack of experience, skill, or ability, or through limitations imposed by the particular method used.

It is first of all essential to definitely establish the exact *purpose* of a standard experimental baking procedure. What information does the average cereal chemist wish to derive from his baking test? Should he expect it to show the optimum bread value that a given flour can produce, as well as the means of producing this result commercially? Or, should it rather indicate that flour property which may be expressed by such terms as "margin of safety", "stability", "intrinsic value", "fermentation tolerance", or "ability to withstand punishment"? Disregarding precise definitions of terms, for present purposes, the preponderance of opinion, insofar as this was revealed at the 1926 annual meeting of the American Association of Cereal Chemists, seems to be overwhelmingly in favor of the latter viewpoint. This viewpoint appears to the writer as the logical basis from which to proceed, for two main reasons. First, the term "optimum results", as applied to test baking, is highly indefinite, elusive, and can never be expressed in terms of universal meaning. Perfection can never be attained in anything, nor is there likely ever to be any agreement as to what constitutes perfection or "optimum results" in bread. Second, the baker, in most instances, is concerned more especially with his flour's stability, or margin of safety, or fermentation tolerance, or whatever he wishes to call it. It therefore appears that any attempt to standardize the baking test should be from this standpoint. It should be borne in mind that the test is to be a test of the flour, itself, and that the results should always be judged and evaluated from that standpoint alone.

Having settled, tentatively at least, upon the object of the test, the next step is obviously to investigate certain already existing methods as may appear to approximate the attainment of this object. In taking this step, certain limitations must be clearly recognized. One is that for the present no hope should be entertained that the personal element in dough manipulation will be eliminated. The complete elimination of this factor is a pleasant dream which will be realized only when certain standard mechanical contrivances are made available to experimental bakers. It must also be understood that any test which will be likely to give satisfaction must be as simple as possible, in order that it will permit of collaborative study. It should not, for the present at any rate, contemplate any extensive revolutionizing of the more important pieces of laboratory equipment. It must necessarily be a method that can be readily and convincingly demonstrated to that rather large group of cereal chemists who may be classed as skeptical or agnostic with regard to the possibility or likelihood of ultimately developing any standard baking procedure whatsoever.

In considering the preliminary selection of a procedure which appears most likely to meet the requirements which have thus far been specified, and since, after all, what is actually wanted is a practical test of a flour's actual *trade value*, it seems logical that first attention should be directed toward the several commercial flour-testing laboratories which have given the most consistently satisfactory service to a variety of customers over a period of years, for the acid test of any practical method is its success or failure under varied commercial and industrial conditions. In the event that several such methods are ascertained, and that they appear to have enjoyed equal success, it then becomes a matter of adopting, tentatively, the one which most nearly meets the following requirements:

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 81.

1. Minimum number of variables.
2. Smallest range of error in replication of tests.
3. Greatest simplicity and ease of operation.
4. Sensible and logical system of scoring and interpretation of results.
5. Good results in the hands of collaborators.
6. Ease of demonstration.
7. Applicability to all types of bread flours.

In accordance with this suggested program, it would seem that the logical order of procedure which should be followed by the writer, acting in the capacity of Associate Referee on Experimental Baking Test for the Association of Official Agricultural Chemists, and chairman of the committee on this activity for the American Association of Cereal Chemists, is:

1. To become acquainted, if possible, with experimental baking procedures practiced by several of the most prominently successful commercial flour-testing laboratories, by visiting these laboratories and consulting with persons in charge.

2. To select, on the basis of this experience, a tentative procedure which may be one of the methods thus studied, or the result of an attempt to combine the best features of several methods. This selection is to be made only after a thorough personal study and practice of each method and a report to the other members of the committee of the American Association of Cereal Chemists.

3. To inaugurate a collaborative study of the tentatively selected method or methods, and to continue such studies until a definite procedure can be agreed upon by the committee.

4. To have a demonstration of the method at that convention of the American Association of Cereal Chemists which immediately follows any agreement of the experimental baking committee of that association.

REPORT ON FAT AND UNSAPONIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES.

By Samuel Alfend[1] (U. S. Food and Drug Inspection Station, St. Louis, Mo.), *Associate Referee.*

The methods recommended by the associate referee this year were subjected to collaborative study on both flour and noodles.

A batch of unbleached flour (A), one of water noodles (B), and one of egg noodles (C), were prepared, and subsamples were placed in Mason jars and sent to the collaborators.

The instructions submitted with the samples are as follows:

FAT.

Method 1.

Direct extraction.—Determine as directed in *Methods of Analysis, A. O. A. C.*, 1925, p. 225, **3** (p. 117, **13**).

Method 2.

Acid hydrolysis.—Determine according to the tentative method, *This Journal*, 1926, **9**: 41.

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[1] Presented by L. C. Mitchell.

UNSAPONIFIABLE MATTER.

Method 1.

Modified Kerr-Sorber.—Extract the lipoids from 5 grams of sample according to the tentative method, *This Journal*, 1926, **9**: 40. To the crude lipoids, add 30 cc. of alcohol and 3 cc. of concentrated potassium hydroxide (1 + 1), and proceed according to the modified Kerr-Sorber method, *This Journal*, 1925, **8**: 441.

TABLE 1.

Collaborative results on fat.

ANALYST	DIRECT EXTRACTION			BLANK	ACID HYDROLYSIS			BLANK
	A	B	C		A	B	C	
	per cent	*per cent*	*per cent*	*gram*	*per cent*	*per cent*	*per cent*	*gram*
Bornmann	0.53	0.12	3.46	0.0030	1.37	2.23	5.05	0.0008
	0.58	0.05	3.47		1.27	2.23	5.09	
Elliott	0.80	0.29	3.60	....	1.26	2.05	4.80	0.0010
	0.80	0.28	3.66		1.23	1.98	4.83	
Bailey	0.69	0.19	3.53	....	1.23	1.99	4.76	0.0020
	0.66	0.28	3.65		1.19	1.90	4.69	
Smith	0.43	0.23	3.17	....	1.26	2.22	4.96	....
	0.42	0.23	3.19		1.36	2.27	5.01	
Mitchell	0.70	0.33	3.43	....	1.17	2.01	4.93	0.0027
	0.75	0.34	3.45		1.12	1.99	5.08	

TABLE 2.

Collaborative results on unsaponifiable matter.

ANALYST	MODIFIED KERR-SORBER METHOD			BLANK	F. A. C. METHOD			BLANK
	A	B	C		A	B	C	
	per cent	*per cent*	*per cent*	*gram*	*per cent*	*per cent*	*per cent*	*gram*
Bornmann	0.18	0.16	0.26	0.0008	0.14	0.13	0.31	0.0005
	0.17	0.17	0.28		0.09	0.11	0.29	
Elliott	0.16	0.15	0.30	....	0.13	0.21	0.32	0.0020
	0.16	0.17	0.29		0.12		0.31	
	0.15	0.20						
	0.15							
Bailey	0.17	0.18	0.31	....	0.14	0.16	0.35	0.0018
	0.14	0.22	0.33		0.12	0.20	0.38	
Smith	0.18	0.21	0.48	....	0.30	0.26	0.50	....
	0.11	0.17	0.40		0.31	0.27	0.47	
Average	0.16	0.18	0.33	....	0.17	0.19	0.36	....
Variation	0.07	0.07	0.22	....	0.21	0.16	0.21	....

Method 2.

F. A. C. Method.—Determine the unsaponifiable matter in the crude lipoids, obtained as in Method 1, by the F. A. C. method, *This Journal*, 1926, **9**: 45.

The results obtained are given in Tables 1 and 2.

COMMENTS OF COLLABORATORS.

L. H. Bailey (*Food Control Laboratory, Bureau of Chemistry, Washington, D. C.*).—The method for fat by direct ether extraction is not considered satisfactory for work with alimentary pastes. Results are too low and variable. Results obtained on fat by the acid hydrolysis method are considered satisfactory. No particular difficulties were encountered in making these tests. The determination of unsaponifiable matter by Method 1 is considered satisfactory, but great care must be exercised to prevent the formation of an emulsion when the sample is first shaken with the ether. There was no tendency to form emulsions by Method 2. Difficulty was experienced at first in pipetting off the petroleum ether extract in Method 2. This difficulty, however, was overcome later, and no great objection was found to the technique given for this method. One method is about as rapid as the other.

J. H. Bornmann (*U. S. Food and Drug Inspection Station, Chicago, Ill.*).—No great difficulties were encountered in either of the fat extraction methods. The acid hydrolysis method requires more work, but the results are obtained in less time than by the old extraction method and there is an astonishing difference in the amount of fat recovered by the two methods.

The modified Kerr-Sorber method for unsaponifiable matter appears to me to be superior to the F. A. C. method with regard to ease of manipulation and time consumed. I believe that it is necessary to boil the solution briskly for at least 20 minutes to insure complete saponification. The final results by the two methods used appear to be practically the same.

R. T. Elliott (*U. S. Food and Drug Inspection Station, Seattle, Wash.*).—The methods designated were followed exactly, with the exception of the fat by acid hydrolysis, in which I found it convenient to use Mojonnier extraction tubes, weighing the flour or the sample directly into the extraction tube and heating with the alcohol and acid, thereby doing away with the transfer of the material from one dish to another after the fat is in a condition to be extracted by ether. I believe that this modification will tend toward the elimination of a possible error in this transfer. I should like to receive comments on this suggestion.

L. C. Mitchell (*U. S. Food and Drug Inspection Station, St. Louis, Mo.*).—Elliott's suggestion of hydrolyzing the sample in the Mojonnier tube directly is time-saving and convenient, and it tends to eliminate a source of error in transferring the hydrolyzed material from the beaker to the extraction tube. I believe it better to add just enough alcohol, after hydrolysis, to make the solution up to the mark on the tube, because adding 10 cc. brings the lower layer into the upper part of the tube and makes it difficult to decant all the ethereal solution from the tube. In the original method of hydrolyzing in a beaker, sufficient liquid is lost by evaporation so that the final solution, on being transferred to the extraction tube, just comes up to the mark. It is difficult to draw definite conclusions as to the proportion of true fat extracted in alimentary pastes by the two methods, because the water noodles do not appear to have been prepared from the unbleached flour from which Sample A was taken.

H. R. Smith (*U. S. Food and Drug Inspection Station, Baltimore, Md.*).—Fat determination by the acid hydrolysis method gave a much higher result than the direct extraction. The blank on the fat by the acid hydrolysis method was zero, indicating that the acid hydrolysis forms ether-soluble material in the sample. In determining the unsaponifiable matter by the modified Kerr-Sorber method, considerable amounts of soap-like material insoluble in petroleum ether were found in each case. In general, the F. A. C. method is preferred.

DISCUSSION OF RESULTS.

It is apparent from the data in Table 1 that the acid hydrolysis method for fat gives considerably higher results than are obtained by the direct extraction method, the discrepancy being most marked in the case of water noodles.

One of the chief functions of the fat determination is to differentiate between cereal products containing eggs and those in which no egg solids are present. Since the direct extraction method is unsuitable for alimentary products, whereas the acid hydrolysis method, which is at present tentative, has been found to be satisfactory, it is desirable that the acid hydrolysis method should be applied to flour also. This advantage alone, however, would not justify the substitution of the acid hydrolysis method for the direct extraction, if the latter method were in wide-spread use, and if many data based upon this method had been collected. Such does not appear to be the case, nor has it been shown that the product obtained by direct extraction is entirely true fat, or that it is all the fat in the flour. Since this method is as empirical as the acid hydrolysis method, it is no more deserving of consideration on that basis. The acid hydrolysis method has the advantage of requiring considerably less time for the determination (though involving more actual work) and of yielding distinctly more uniform results in the hands of the various collaborators.

In view of the advantages cited, the associate referee would recommend that the acid hydrolysis method be selected if the association is to have but one method for the determination of fat in flour.

Since the slight modification suggested by Elliott and recommended by Mitchell, who tried it on the suggestion of the associate referee, is chiefly one of convenience, and does not greatly affect the accuracy of the method, the associate referee does not feel justified in recommending a change in the directions unless the method is subjected to further collaborative study.

The average results for unsaponifiable matter are practically the same by the modified Kerr-Sorber and the F. A. C. methods, although the former yields appreciably more uniform results. The collaborators are not agreed as to the relative merits of these methods, particularly in regard to time and convenience of operation.

As a means for distinguishing between cereal products containing egg solids and those containing no egg solids, the determination of unsaponifiable matter hardly appears to be of sufficient value at this time to justify inclusion in the methods of this association. If, however, the association agrees with the general referee who recommended this determination for study, that the chapter on Cereal Foods in its book of methods[1] should "eventually include all worthy known methods of analysis that may have value to any chemist interested in cereal analysis and investigations and to develop these methods to their highest state of perfection", then the associate referee would favor the adoption of the F. A. C. method as a tentative method until a superior method has been worked out and tested. The collaborative study carried on this year has not verified in any decided fashion the claims of the authors of the modified Kerr-Sorber method regarding its simplicity and brevity. The F. A. C. method is by far the most widely used, and it has now been adopted by the association for oils, fats, and waxes in preference to the modified Kerr-Sorber method.

It will be noted that the determination of unsaponifiable matter was made on the crude lipoids obtained by Hertwig's neutral extraction method[2], instead of on the residue of the ethyl ether or petroleum ether extract after the removal of the ether. Hertwig has shown that direct extraction with ether does not obtain all the ether-soluble and fat-like substances from wheat flour and alimentary pastes. Hertwig and Bailey[3] found that the neutral extraction method yields a greater amount of unsaponifiable matter than does the acid hydrolysis method, the Rask-Phelps alkaline extraction[4], or the direct ether extraction.

RECOMMENDATIONS[5].

It is recommended—

(1) That the acid hydrolysis method for fat be adopted as an official method for flour and for alimentary pastes.

(2) That the F. A. C. method for unsaponifiable matter carried out on the lipoids extracted by the tentative method be adopted as a tentative method for flour and for alimentary pastes.

REPORT ON BREAD ANALYSIS.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

It was the desire of the associate referee to secure collaborative study of the methods of bread analysis that have been considered by this association. The study included two methods for the determination of each of the following: total solids, ash, and lipoids.

The American Institute of Baking and six branch laboratories of the

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[1] *Methods of Analysis, A. O. A. C.*, 1925.
[2] *This Journal*, 1923, 6: 508.
[3] *Ibid.*, 1926, 9: 122.
[4] *Ind. Eng. Chem.*, 1926, 17: 189.
[5] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 82.

Bureau of Chemistry were invited to collaborate. Owing to changes in personnel and pressure of other work, only one of the branch laboratories was able to make a report. A report from the American Institute of Baking covered only a portion of the work.

It is greatly regretted that better collaboration was not secured, but it was too late to obtain other collaborators when the associate referee was notified of the inability of some of the laboratories to make a report.

MOISTURE.

The collaborators were asked to procure a fresh loaf of bread, slice it, and dry the slices until air dry, then grind the broken slices and make the different determinations upon the ground material. The total solids in the loaf were to be calculated to the original moisture basis. In three cases, noted in the report, the total solids are given on the air-dried samples instead of on the original loaf. Close agreement in results was obtained at the Bureau of Chemistry in Washington on two separate samples prepared and dried by the three drying methods indicated, namely the vacuum method using the temperature of boiling water, 98.5°–99°C. and a pressure of less than 1 inch[1]; the air-oven method at a temperature of 130°C.[2]; and the distillation method of Bidwell and Sterling[3].

Greater variation was shown in results obtained by the vacuum-oven and the 130°C. methods from the Denver laboratory where the boiling temperature of the water surrounding the vacuum oven was only 94°C. than from the other laboratories. Not all of the moisture was removed at this low temperature. Some darkening of the sample was noted in the case of drying in the air oven at 130°C. In the report from the American Institute of Baking the percentages of solids are given on the air-dried samples and not on the original bread. The Institute also sent in results obtained by drying the sample in an air oven for 1 hour at 150°C. The percentage loss was approximately 0.1 per cent greater than when dried in an air oven for 1 hour at 130°C.

ASH.

The results obtained by determining ash by the official method for the determination of ash in wheat flour and by the glycerol-alcohol method proposed by Hertwig and Bailey[4] were quite similar; they indicate that equally accurate results can be obtained by either method. Under the same conditions of combustion, when alcohol and glycerin are used, the ash is lighter in color and more fluffy than when the official method is used.

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[1] *This Journal*, 1926, 9: 42.
[2] *Ibid.*, 40.
[3] *Ibid.*, 30.
[4] *Cereal Chem.*, 1925, 2: 38.

FAT—LIPOIDS.

Two methods were studied for determining the fat-like substances in bread: the "fat" method, which is now tentative for baked cereal products and the "lipoid" method, which is now tentative for other cereal products. The results indicate that higher values are obtained when the lipoid method is used. It would seem logical to adopt this method also for baked cereal products, and then have uniform methods for lipoids in all cereal products.

Owing to the small number of collaborative results obtained this year, the associate referee does not feel justified in recommending any changes in existing methods, but suggests a continuation of this year's studies for another year[1].

It is also suggested that in the future consideration be given by the association to the development of methods for determining the amounts of the characteristic constituent of special breads, such as the quantity of raisins in raisin bread, the quantity of rye in rye bread, and the quantity of milk in milk bread.

TABLE 1 (Note).
Collaborative results.
(Averages of close-agreeing duplicates.)

COLLABORATOR	TOTAL SOLIDS			ASH		FAT—LIPOIDS	
	Vacuum Method	Air-Oven Method	Distillation Method	Tentative Method[*]	Alcohol-Glycerol Method[†]	Tentative Method[‡]	Tentative Method[¶]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Leonard Feldstein Food and Drug Inspection Laboratory, Denver, Colo.	69.25	68.85	....	2.26	2.28	3.86	4.30
C. E. Goodrich Bureau of Chemistry Washington, D. C.	63.19	63.18	63.26	2.47	2.50	4.58	4.96
L. H. Bailey	62.22	62.26	62.26	2.26	2.33	4.71	5.66
W. C. Luckow (a)	93.09[§]	...	....	...	.	3.87	...
C. Conrad (b) American Institute of Baking, Chicago, Ill.	....	92.55[§]	93.03[§]	1.96	1.94	....	....

[*] *This Journal*, 1926, **9**: 42.
[†] *Cereal Chem.*, 1925, **2**: 38.
[‡] *Methods of Analysis, A. O. A. C.*, 1925, 231.
[¶] *Ibid.*, 233.
[§] These results are based on the air-dried sample and not on the original moisture basis.

NOTE.—The moisture results obtained by distillation as shown in this table were made by W. F. Sterling, Bureau of Chemistry, and C. Conrad, American Institute of Baking.

No report on specific gravity and alcohol was given by the referee.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 80.

REPORT ON VINEGAR.

By J. O. Clarke (Food and Drug Inspection Station, New York, N. Y.), *Referee.*

In part compliance with the recommendation of the referee for last year, methods for total ash, phosphoric acid, non-volatile reducing substances, and sulfates were studied. No work on glycerol or polarization was undertaken.

A sample of generator-run vinegar containing 10 mg. of added sulfur trioxide was submitted to collaborators, who were requested to make the following determinations:

Total ash,
Water-soluble ash,
Water-soluble phosphoric acid,
Water-insoluble phosphoric acid,
Total phosphoric acid,
Non-volatile reducing substances, and
Sulfates.

Total and water-soluble ash was to be determined by both Methods A and B[1]; water-soluble and insoluble phosphoric acid was to be determined by Methods 8 and 9[1] on the ash from both Methods A and B; total phosphoric acid was to be determined on ash by Method B; and non-volatile reducing substances and sulfates by the tentative method of the association[2]. Sulfates were to be determined by the following method:

Ash 100 cc. of the sample in a platinum dish at a low red heat, using Method 5 (b) to incinerate all carbon particles. Dissolve the ash in about 10 cc. of approximately normal hydrochloric acid. Dilute to about 100 cc. with distilled water, heat to boiling, add 10 cc. of hot barium chloride solution (1 gram 100 cc.) drop by drop, and continue as outlined in the tentative method, par. 24[3].

The referee wishes to express his appreciation to the heads of the collaborating laboratories and to the following chemists who did the analytical work:

C. A. Greenleaf, Bureau of Chemistry, Washington, D. C.
R. S. Roe, Bureau of Chemistry, Chicago, Ill.
L. Katz, Bureau of Chemistry, New York, N. Y.
H. R. Smith, Bureau of Chemistry, Baltimore, Md.
J. Calloway, Jr., Bureau of Chemistry, Savannah, Ga.
J. Fitelson, Bureau of Chemistry, New York, N. Y.

L. Katz also did additional work on this subject in the laboratory of the New York Station of the Bureau of Chemistry.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 325.
[2] *Ibid.*, 326.
[3] *Ibid.*, 329.

TABLE 1.

Collaborative results on vinegar.

(Phosphoric acid expressed in mg. per 100 cc., all other results as grams per 100 cc.)

DETERMINATIONS	GREENLEAF		ROE		KATZ		SMITH		CALLOWAY		FITELSON		LOWEST	HIGHEST	AVERAGE
Total Ash—Method 5 (a)........	0.35	0.35	0.38	0.38	0.37	0.37	0.35	0.36	0.35	0.35	0.36	0.36	0.35	0.38	0.36
Total Ash—Method 5 (b).......	0.37	0.37	0.39	0.40	0.35	0.36	0.35	0.37	0.34	0.34	0.38	0.38	0.34	0.40	0.37
Water-soluble Ash—Method 5 (a)	0.31	0.31	0.33	0.33	0.33	0.33	0.32	0.32	0.31	0.31	0.31	0.31	0.31	0.33	0.32
Water-soluble Ash—Method 5 (b)	0.35	0.34	0.34	0.36	0.31	0.31	0.31	0.33	0.30	0.30	0.33	0.33	0.30	0.36	0.33
Phosphoric Acid—Water-soluble P_2O_5—Method 5 (a)*..........	11.8	11.7	13.1	13.9	11.7	11.5	9.0	8.5	11.7	11.5	10.1	12.8	8.5	13.9	11.4
Phosphoric Acid—Water-soluble P_2O_5—Method 5 (b)*..........					11.6	11.8	8.6	8.7	12.7	12.9	13.6	14.2	8.6	14.2	11.7
Phosphoric Acid—Water-insoluble P_2O_5—Method 5 (a)*... ...	14.8	14.3	17.0	16.6	11.4	11.5	12.9	13.3	9.4	9.5	12.7	10.1	9.4	17.0	12.8
Phosphoric Acid—Water-insoluble P_2O_5—Method 5 (b)*..........					11.4	11.3	13.3	12.9	7.4	7.3	9.5	9.1	7.3	13.3	10.3
Phosphoric Acid—Total P_2O_5, Method 5 (b)*..............	23.9	23.8	26.9	26.5	22.2	22.2	22.4	21.6	19.9	20.2	22.3	23.0	19.9	26.9	22.9
Non-volatile Reducing Substances	0.43	0.43	0.39	0.40	0.40	0.40	0.41	0.42	0.42	0.43	0.39	0.41	0.39	0.43	0.41
Sulfates (SO_3)—															
Tentative Method....	16.3	15.9	16.5	16 7	16.4	16.5	15.1	15.7	17.8	18.1	16.5	16.4	15.1	18.1	16.5
Proposed method.....	19.7	18.4	16.5		17.3	17.2	16.8	16.8	18.1	18.4	17.0	17.0	16.5	19.7	17.6

* Ashed by method noted.

DISCUSSION.

TOTAL ASH.

Total ash was determined in the writer's laboratory on four samples of cider vinegar by Method B, the incinerating being done at the lowest possible heat (barely visible dark red) and on the same samples at a bright red heat. The results obtained by L. Katz, analyst, expressed as grams per 100 cc., are given in Table 2.

TABLE 2.

Sample Number	44990	5490	24668	8539
Total ash (low heat)	0.43	0.37	0.31	0.28
Total ash (high heat)	0.42	0.36	0.30	0.28

The difference between the results obtained at low heat and those obtained at high heat was very small. In a similar manner, another sample was ashed at four different temperatures, varying from a barely visible red heat to bright red, and the result at the lowest temperature was only 0.02 gram per 100 cc. higher than at the highest heat. These results would tend to indicate that the temperature of ashing is not of the greatest importance, but this conclusion should be confirmed by future work in which definite temperature measurements are used.

Collaborative results on ash obtained by using Method A are good. With Method B the lowest result is 0.34 and the highest 0.40. Last year's collaborative work on this determination yielded slightly better results by Method B, but neither method produced very good results. Two years' work, therefore, tends to indicate that the methods for ash need revision of a fundamental character.

Soluble ash naturally follows total ash, and the same criticism would apply.

PHOSPHORIC ACID.

A study was made in the writer's laboratory by L. Katz of the influence of the temperature of ashing on the subsequent determination of soluble and insoluble phosphoric acid. Using the ash reported in Table 2, soluble and insoluble phosphoric acid was determined. The results, expressed as mg. per 100 cc., are given in Table 3.

TABLE 3.

Sample Number	44990	5490	24668	8539
Soluble P_2O_5 (low heat)	5.8	7.8	10.1	11.7
Soluble P_2O_5 (high heat)	11.2	11.5	12.6	13.6
Insoluble P_2O_5 (low heat)	23.3	9.3	9.9	8.2
Insoluble P_2O_5 (high heat)	18.2	5.6	7.8	6.5
Total sum of soluble and insoluble P_2O_5 (low heat)	29.1	17.1	20.0	19.9
Total sum of soluble and insoluble P_2O_5 (high heat)	29.4	17.1	20.4	20.1

There is uniformly a larger quantity of soluble phosphoric acid obtained when a higher temperature is used in ashing; however, the sum of soluble and insoluble phosphoric acid is not affected. This difference throws a doubt on the value of separate determinations of soluble and insoluble phosphoric acid. Doubtless the difference is caused by an incipient fusion of the ash material, converting some insoluble phosphoric acid to the soluble form.

The collaborative results show a wide but corresponding difference in the value reported for both soluble and insoluble phosphoric acid. While the results for total phosphoric acid are not so close as could be desired they are much better than those for either soluble or insoluble phosphoric acid. Further work should be done on this subject in connection with the determination on ash with the possibility of dropping the existing method for soluble and insoluble, and substituting therefor a method for total phosphoric acid.

NON-VOLATILE REDUCING SUBSTANCES.

Collaborative results, which showed maximum and minimum values of 0.43 and 0.39 among six collaborators, were much better than last year, and they warrant the final adoption of this method as official. This is especially gratifying in view of the results on collaborative samples in 1923. The method for total reducing substances was made official, final action last year. Volatile reducing substances are the difference between total reducing substances and non-volatile reducing substances; therefore, the method or directions for calculating volatile reducing substances should also be made official.

SULFATES.

Two methods were studied collaboratively, the tentative method and a proposed method based on ashing the material before precipitation of barium sulfate. Neither method yielded especially good results, although the tentative method was the better. This method should be further studied before it is recommended for adoption as official.

RECOMMENDATIONS[1].

It is recommended—

(1) That methods for total and soluble ash be further studied.

(2) That the subject of phosphoric acid be studied with the view to possible substitution of a method for total phosphoric acid for the present methods for soluble and insoluble phosphoric acids.

(3) That Method **15**, non-volatile reducing substances, and Method **16**, volatile reducing substances, be adopted as official (final action).

(4) That Method **18**, glycerol, Method **23**, polarization, and Method **24**, sulfates, be further studied.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 75.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES.

By J. W. SALE (Bureau of Chemistry, Washington, D. C.), *Referee.*

In accord with last year's recommendations, additional work was done on the Folin and Denis[1] rapid colorimetric method for the determination of vanillin and on the analysis of non-alcoholic flavors.

EFFECT OF ADDED CARAMEL ON DETERMINATION OF VANILLIN BY FOLIN AND DENIS METHOD.

It has been observed by J. B. Wilson and the writer[2] that caramel interferes with the determination of vanillin by the Folin and Denis method. These authors analyzed four commercial samples of caramel by the method in question and found that apparently they contained 4.32, 4.06, 0.78, and 0.58 per cent of vanillin, respectively, when they were dissolved in 47.5 per cent of alcohol. When the method was applied to a solution of caramel in 95 per cent of alcohol, negative results were obtained.

In order to determine whether or not the usual quantity of caramel present in artificially colored imitation vanilla extracts introduces an appreciable error when this method is used, C. H. Badger, at the request of the referee, prepared and analyzed nine synthetic samples that simulated true vanilla extract in appearance and flavor. They consisted of 0.5 per cent of vanillin, 0.8 per cent of caramel, about 20 per cent of alcohol, and water. The addition of 0.8 per cent of caramel gave the synthetic solutions the appearance of a rather dark-colored true vanilla extract. The samples of caramel used represented nine brands manufactured by eight firms. They were first made up into a 5 per cent solution, 47.5 per cent by volume of alcohol being used as a menstruum. Another synthetic sample had the same composition as those just described except that it contained no caramel. All ten samples were analyzed for content of vanillin by the method of Folin and Denis. The method was applied also to the 5 per cent caramel solutions. All results are set forth in Table 1.

COMMENT ON TABLE 1.

The colors produced by caramel were generally of a different shade of blue than those produced by vanillin, but they were sufficiently like the true color to be mistaken for it by an inexperienced analyst. In order to obtain correct results by the Folin and Denis method, it was found necessary to make up the dilute standard vanillin solution each day from a 1 per cent alcoholic solution, because dilute vanillin solutions deteriorate on standing overnight. Comparison of colors should

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[1] *This Journal*, 1925, 8: 688.
[2] *Ind. Eng. Chem.*, 1926, 18: 283.

TABLE 1.

Interference by caramel with determination of vanillin.

SAMPLE NO.	BRANDS OF CARAMEL USED	APPARENT VANILLIN IN CARAMEL	CARAMEL PRESENT IN SYNTHETIC SAMPLES*	VANILLIN PRESENT IN SYNTHETIC SAMPLES	VANILLIN FOUND IN SYNTHETIC SAMPLES	ERROR
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	A	1.28	0.8	0.5	0.502	+0.002
2	B	1.37	0.8	0.5	0.502	+0.002
3	C	1.17	0.8	0.5	0.498	−0.002
4	D	1.17	0.8	0.5	0.499	−0.001
5	E	1.96	0.8	0.5	0.502	+0.002
6	F	2.14	0.8	0.5	0.505	+0.005
7	G	2.21	0.8	0.5	0.497	−0.003
8	H	0.58	0.8	0.5	0.490	−0.010
9	I	1.77	0.8	0.5	0.506	+0.006
10	..	....	0.0	0.5	0.498	−0.002

* Part commercial caramel per 100 parts of synthetic sample.

be made immediately after the addition of reagents to the sample and standards as the standards tend to become cloudy, and this condition vitiates the results.

Inspection of Table 1 shows that while all the brands of caramel gave a strong test for vanillin, the proportion of caramel ordinarily used in imitation vanilla extracts is so small that the figures for vanillin are not materially affected. In view of these data the referee believes that this method may now be adopted as an alternative official method.

POLARISCOPE METHOD FOR ESSENTIAL OILS.

Last year, the referee reported satisfactory results obtained by J. B. Wilson, who used the polariscope method for the determination of oils of lemon and orange in corn oil, cottonseed oil, and mineral oil. However, it was deemed advisable to extend the experimetal work, and this was done. Polariscope readings were made by collaborators other than Wilson on the samples reported last year, and new samples were prepared and readings made by three collaborators. The new samples consisted of oils of lemon, orange, and limes dissolved in corn oil, cottonseed oil, peanut oil, and mineral oil. The results obtained are given in Table 2, which should be considered in connection with Table 5[1] reported last year. It will be observed that 74 samples have been analyzed by this method, and since each polariscope reading reported in the tables is the average of five readings, a total of over 900 polariscope readings have been made by the collaborators.

It is believed that the data in Table 5 of last year's report and in Table 2 of this report warrant the adoption of the polariscope method as a tentative method. The detailed procedure has been published[2].

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[1] *This Journal*, 1926, 9: 455.
[2] *Ibid.*, 1927, 10: 43.

TABLE 2.

Determination of essential oils in vegetable and mineral oils.

SAMPLE NO.*	MENSTRUUM	KIND OF ESSENTIAL OIL	POLARISCOPE READINGS (°V.—20°C.)††			FACTOR	ESSENTIAL OIL FOUND % BY VOLUME§§				ESSENTIAL OIL PRESENT, % BY VOLUME	AVERAGE ERROR
			Sale	Mix	Wilson		Sale	Mix	Wilson	Average		
												per cent
1	Corn Oil	Lemon†			+ 0.6	3.4					0.00	
3	"	"		+11.7				3.27			3.33	− 1.8
4	"	"		+17.9				5.09			5.00	+ 1.8
5	"	"		+24.2				6.94			6.67	+ 4.0
36	"	" ‡	+11.0	+10.5	+10.6		3.06	2.91	2.94	2.97	3.33	−10.8
37	"	"	+16.8	+16.9	+17.1		4.77	4.79	4.85	4.80	5.00	− 4.0
38	"	"	+28.0	+28.2	+28.0		8.06	8.12	8.06	8.08	8.33	− 3.0
1	"	Orange§			+ 0.6	5.4					0.00	
6	"	"		+ 8.5				1.46			1.67	−12.5
7	"	"		+17.9				3.20			3.33	− 3.9
8	"	"		+26.5				4.80			5.00	− 4.0
9	"	"		+36.0				6.56			6.67	− 1.6
39	"	" **	+18.6	+18.5	+18.9		3.33	3.31	3.39	3.34	3.33	+ 0.3
40	"	"		+27.6				5.00			5.00	± 0.0
1	"	Limes††			+ 0.6	2.0					0.00	
41	"	"	+ 6.5	+ 6.6	+ 6.6		2.95	3.00	3.00	2.98	3.33	−10.5
42	"	"	+10.7	+10.2	+10.5		5.05	4.80	4.95	4.93	5.00	− 1.4
43	"	"	+18.0	+17.9	+18.0		8.70	8.65	8.70	8.68	8.33	+ 4.2
10	Cottonseed Oil	Lemon§			− 0.3	3.7					0.00	
13	"	"		+18.5				5.08			5.00	+ 1.6
14	"	"		+24.7				6.76			6.67	+ 1.3
15	"	"		+31.1				8.49			8.33	+ 1.8
10	"	" †			− 0.3						0.00	
44	"	"	+11.6	+11.5	+11.5		3.22	3.19	3.19	3.20	3.33	− 3.9
45	"	"	+17.4	+17.6	+17.5		4.78	4.84	4.81	4.81	5.00	− 3.8
10	"	" ‡			− 0.3						0.00	
46	"	"	+11.5	+11.4	+11.2		3.19	3.16	3.11	3.15	3.33	− 5.7
47	"	"	+17.5	+17.5	+17.5		4.81	4.81	4.81	4.81	5.00	− 3.8
48	"	"	+29.0	+29.4	+29.4		7.92	8.03	8.03	7.99	8.33	− 4.1
10	"	Orange†			− 0.3	5.7					0.00	
16	"	"		+ 9.1				1.65			1.67	− 1.2
17	"	"		+18.6				3.32			3.33	− 0.3

18	Cottonseed Oil	Orange†		+27.9				4.95			5.00	− 1.0
19	"	"		+37.7				6.67			6.67	± 0.0
20	"	"		+41.4				7.32			8.33	−12.1
49	"	" §	+19.3	+19.3	+19.1		3.44	3.44	3.40	3.43	3.33	+ 3.0
50	"	"	+28.9	+29.0	+28.9		5.12	5.14	5.12	5.13	5.00	+ 2.6
51	"	" **	+18.9	+18.7	+19.1		3.37	3.33	3.40	3.37	3.33	+ 1.2
52	"	"	+27.8	+28.7	+28.0		4.93	5.09	4.97	5.00	5.00	± 0.0
10	"	Mandarin Orange			− 0.3	4.1					0.00	
53	"	"	+16.1	+16.4	+16.6		4.00	4.07	4.12	4.06	4.00	+ 1.5
54	"	"	+24.3	+24.5	+24.4		6.00	6.05	6.02	6.02	6.00	+ 0.3
10	"	Limes††			− 0.3	2.3					0.00	
55	"	"	+ 7.7	+ 7.5	+ 7.8		3.48	3.39	3.52	3.46	3.33	+ 3.9
56	"	"	+11.2	+11.1	+11.2		5.00	4.96	5.00	4.99	5.00	− 0.2
57	"	"	+19.0	+18.8	+18.9		8.39	8.30	8.35	8.35	8.33	+ 0.2
58	Peanut Oil	Lemon†	+ 0.2		+ 0.2	3.6					0.00	
59	"	"	+ 5.7	+ 5.5	+ 6.1		1.53	1.47	1.64	1.55	1.67	− 7.2
60	"	"	+12.0	+11.9	+12.2		3.28	3.25	3.33	3.29	3.33	− 1.2
61	"	"	+17.6	+17.3	+17.4		4.85	4.75	4.78	4.79	5.00	− 4.2
62	"	"	+29.4	+29.3	+29.5		8.11	8.08	8.14	8.11	8.33	− 2.6
63	"	" §	+ 6.4	+ 6.4	+ 6.4		1.72	1.72	1.72	1.72	1.67	+ 3.0
64	"	"	+12.3	+12.3	+12.3		3.36	3.36	3.36	3.36	3.33	+ 0.9
65	"	"	+18.9	+18.7	+18.7		5.19	5.14	5.14	5.16	5.00	+ 3.2
66	"	"	+31.6	+31.7	+31.5		8.72	8.75	8.69	8.72	8.33	+ 4.7
21	Mineral Oil	" ‡			+ 5.5	3.6					0.00	
67	"	"	+17.6	+17.7	+17.6		3.41	3.44	3.41	3.42	3.33	+ 2.6
68	"	"	+23.6	+23.5	+23.5		5.11	5.08	5.08	5.09	5.00	+ 1.8
69	"	"	+34.6	+34.6	+34.2		8.21	8.21	8.10	8.17	8.33	− 1.9
21	"	Orange†			+ 5.5	5.7					0.00	
31	"	"		+15.8				1.82			1.67	+ 9.0
32	"	"		+24.7				3.40			3.33	+ 2.1
34	"	"		+37.9	+38.0			5.74	5.76	5.75	6.67	−13.8
70	"	" **	+24.3	+24.1	+24.3		3.15	3.12	3.15	3.14	3.33	− 5.7
71	"	"	+33.7	+33.3	+33.7		5.00	4.92	5.00	4.97	5.00	− 0.6
21	"	Limes††			+ 5.5	2.3					0.00	
72	"	"	+13.6	+13.5	+13.5		3.61	3.56	3.56	3.58	3.33	+ 7.5
73	"	"	+17.4	+17.0	+17.3		5.30	5.12	5.26	5.23	5.00	+ 4.6
74	"	"	+25.1	+25.1	+25.0		8.73	8.73	8.69	8.72	8.33	+ 4.7

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* Samples bearing numbers 34 and less are the same as those bearing same numbers in Table 5 in Report on Flavors and Non-alcoholic Beverages 1925.
† Hand-pressed Italian oil.
‡ Same as Sample 2 except very old.
§ California oil.
** California distilled oil.
†† Distilled oil.
‡‡ Each figure is average of five readings; 200 mm. tubes used.
§§ For method of calculation, see description of polariscope method in body of report.

The method of obtaining the factors referred to above is fully described in the Report on Flavors and Non-Alcoholic Beverages for 1925[1]. Further experimental work should be done with a view to determining whether or not the rotation of the menstruum in an unknown sample can be readily determined, as for example, by distilling off the essential oil, drying the menstruum, and taking readings of it in a polariscope.

RECOMMENDATIONS[2].

It is recommended—

(1) That the Folin and Denis rapid colorimetric method for the determination of vanillin in vanilla extract and its imitations, described in the referee's report for 1924, be adopted as an alternative official method (final action).

(2) That the polariscope method for the determination of oils of lemon, orange, and limes, in corn oil, cottonseed oil, peanut oil, and mineral oil, described in this report, be adopted as a tentative method.

(3) That the steam distillation method for the determination of essential oils in non-alcoholic flavors, described in the report of the referee for 1925, be subjected to further tests.

REPORT ON MEATS AND MEAT PRODUCTS.

By R. H. Kerr (Bureau of Animal Industry, Washington, D. C.),
Referee.

It was found impossible to carry out the recommendation for collaborative study and test of the method for nitrites or to do collaborative work of any kind owing to a lack of collaborators. The referee was also unable to find a suitable associate referee for work on analytical methods, so no one has been recommended for appointment. However, it is believed that an Associate Referee on Analytical Methods should be appointed as soon as possible. As in the preceding year, no report was received from W. S. Ritchie, Associate Referee on Separation of Meat Proteins. The referee understands that Ritchie has not been entirely idle, but that his work has not progressed sufficiently to justify a report.

Work in the writer's laboratory on the determination of total nitrogen in meats and meat products by the Kjeldahl-Gunning-Arnold method shows that the time of digestion specified is far in excess of that necessary. It is recommended, therefore, that this method[3] be changed to read as follows:

> . . . . in the Kjeldahl-Gunning-Arnold method for 1 hour after the mixture has become clear.

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[1] *This Journal*, 1926, 9: 446.
[2] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 76.
[3] *Methods of Analysis, A. O. A. C.*, 1925, 237, 6.

In this connection, attention is called to the fact that the use of an electric digestion apparatus, which is becoming decidedly common, introduces a problem that ought to be given careful consideration by the Referee on Nitrogen.

Attention is called to the fact that while the association has methods for the determination of total moisture and for the determination of nitrogen, there is no official method for the determination of added water in meats and meat products, notwithstanding the fact that added water is one of the determinations that an official analyst is very frequently called upon to make. The four to one ratio between water and protein is now so well established and universally applied that any collaborative work to further establish its reliability would appear to be a superfluous formality. The following, therefore, is recommended[1] for adoption as a tentative method:

ADDED WATER.

(Applicable to sausage and other prepared meat products.)

Determine total moisture by drying in air at 100°C. for 16–18 hours, or under a vacuum of not less than 100 mm. for 5 hours. Determine nitrogen in accordance with Section **6**. Calculate protein by multiplying the nitrogen found by the factor 6.25. Calculate normal water by multiplying protein by the factor 4.0. Subtract the normal water as found by calculation from the actual moisture found by drying. Report the difference as added water.

It is further recommended that the methods for soluble and insoluble nitrogen, Chapter XVII, Sections **25** and **26**[2]; for coagulable nitrogen, Section **27**; proteose, peptone, and gelatin nitrogen, Section **28**; meat bases, Section **29**; amino nitrogen, Sections **31**, **32**, **33**, and **34**; total soluble phosphorus, Section **35**; separation of soluble inorganic and organic phosphorus, Section **36**, and for soluble phosphorus in blood, brain, and glandular organs, Section **37**, be deleted, for the reason that these methods are intended rather for research on the composition of meat than for practical application and are not, therefore, of particular value to members of the association.

Attention is directed to the fact that the tentative method for nitrites, Section **14**, directs that nitrites be determined in a suitable aliquot, as directed in Chapter VIII, Section **15**. The method was recommended in this form in order that there might be one method only for the determination of nitrites in *Methods of Analysis*. The reagents specified for the determination of nitrites in that method, Chapter VIII, Section **14**, are not wholly satisfactory. The referee prefers the single-solution nitrite reagent specified in "Standard Methods of Water Analysis", published by the American Public Health Association to the two-solution reagent specified in the official method. The method for preparing the standard solution of nitrite by precipitation of silver nitrite is not satisfactory. It is much simpler and more accurate to determine the nitrite strength of a solution of sodium nitrite and make the appropriate dilution. It is recommended that this suggestion be referred to the Referee on Waters, Brine, and Salt for consideration and appropriate action[1].

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 77.

[2] The numbers in bold type refer to sections of the chapters as given, *Methods of Analysis*, *A. O. A. C.* 1925.

No report on the separation of meat proteins was given by the associate referee.

No report on gelatin was given by the referee.

No report on spices and other condiments was given by the referee.

REPORT ON CACAO PRODUCTS.

By E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.), *Referee.*

Studies of microscopical methods for the estimation of shell were continued, and progress is reported but no formal report by the associate referee can be presented at this time.

No report can be presented on the subject of fiber in cacao products and the recommendation of last year is repeated.

RECOMMENDATIONS[2].

The following recommendations relating to cacao products in general are offered.

It is recommended—

(1) That the study of methods for the estimation of shell in cacao products be continued.

(2) That studies be made of methods for the determination of casein, sucrose, and lactose in cacao products.

(3) That the study of the subject of crude fiber in cacao products, as outlined last year[3], be continued.

(4) That the study of methods for the detection of foreign fats in cacao products be continued.

In addition to the general report of progress of the Referee on Cacao Products, he also submits the following report on the determination of fat in cacao products.

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[1] For report of Sub-committee C and action of the association, see *This Journal*, 1927, 10: 77.
[2] *Ibid.*, 78.
[3] *This Journal*, 1926, 9: 461.

DETERMINATION OF FAT IN CACAO PRODUCTS.

By E. M. BAILEY.

Last year several methods for the determination of fat in cacao products were reported upon, and the Lepper and Waterman procedure[1] was recommended for adoption as an official method.

A modification of the old official method gave very satisfactory results, however, in the hands of the several collaborators, and it was recommended for further study. During the past year a report has been submitted by one additional collaborator, M. A. Pozen, Chief Chemist, Schwarz Laboratories, Inc., New York, the analyses being made by G. W. Enners. The analytical results were accompanied by the following comments:

> In general the agreement between the two methods (Lepper and Waterman and the proposed modification of the old method), is good. Four hours' extraction is not always sufficient by the last-named method. The sample was mixed with ignited sea sand instead of asbestos. In our opinion, the proposed modification is satisfactory and sufficiently desirable to warrant its adoption as an official method.

The combined results for the past two years may be summarized as follows:

Determinations of fat in cacao products.

	LEPPER AND WATERMAN METHOD	PROPOSED MODIFICATION
	per cent	*per cent*
SAMPLE D.		
(Five collaborators in 1925.)		
Maximum	52.03	52.05
Minimum	51.54	51.41
Average	51.81	51.74
(One collaborator in 1926.)		
	51.13	51.23
Maximum variation	0.90	0.82
SAMPLE 6 CS.		
(Five collaborators in 1925.)		
Maximum	36.86	36.78
Minimum	36.74	36.55
Average	36.80	36.69
(One collaborator in 1926.)		
	36.65	36.68
Maximum variation	0.21	0.23
SAMPLE 8 DM.		
(Five collaborators in 1925.)		
Maximum	40.14	40.81
Minimum	39.79	39.59
Average	40.02	40.16
(One collaborator in 1926.)		
	39.50	39.60
Maximum variation	0.64	1.22

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[1] *This Journal*, 1926, 9: 46.

The experimental material this year was from the same supply as that submitted to collaborators in 1925, but it had been kept in the meantime with no special precautions to prevent changes in composition. The significant comparisons are between the two methods and not necessarily between the results obtained this year and last. However, the new results introduce no variations that indicate any serious change in the samples.

The variation (1.22 per cent), noted in sample 8 DM, modified method, becomes 0.61 per cent if a single result obtained last year is excluded.

While it is generally undesirable to adopt two official methods for the same determination, yet continuous extraction possesses some advantages over repeated washings with solvents and is preferred by some analysts. The results obtained by the modification proposed are satisfactory as compared with the Lepper-Waterman method; and also, so far as comparison has been made, with the Roese-Gottlieb method. It seems justifiable to recognize this procedure and suggest it as a tentative method. The details of the method have been published[1].

RECOMMENDATIONS[2].

It is recommended—

(1) That the Lepper-Waterman method for the determination of fat in cacao products be adopted as the official method (final action).

(2) That the old official method for the determination of fat[3] be deleted (first action).

(3) That the modification of the old official method, as herein described and as previously outlined, be adopted as a tentative method for the determination of fat in cacao products.

No report on microscopical methods was given by the associate referee.

No report on crude fiber was given by the associate referee.

No report on cacao butter was given by the associate referee.

No report on naval stores was given by the referee.

No report on turpentine was given by the associate referee.

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[1] *This Journal*, 1927, **10**: 42.

[2] For report of Sub-committee C and action of the association, see *This Journal*, 1927, **10**: 78.

[3] *Methods of Analysis, A. O. A. C.*, 1925, 345, 14.

THIRD DAY.

WEDNESDAY—MORNING, AFTERNOON, AND EVENING JOINT SESSIONS.

The 20th anniversary of the passage of the Federal food and drugs act was commemorated by joint meetings of the Association of Official Agricultural Chemists, the Association of Dairy, Food and Drug Officials and the Association of Feed Control Officials, on October 20, in the Ball Room of the Willard Hotel.

Addresses were given in the order listed by the following:

Hon. R. W. Dunlap, Assistant Secretary of Agriculture.
Dr. W. W. Randall, President, Association of Official Agricultural Chemists.
Mr. Thomas Holt, President, Association of Dairy, Food and Drug Officials.
Mr. F. D. Fuller, President, Association of Feed Control Officials.
Hon. W. M. Jardine, Secretary of Agriculture.
Dr. C. A. Browne, Chief, Bureau of Chemistry.
Charles Wesley Dunn, Counsel, American Grocery Specialty Manufacturers' Association.
Mrs. Walter McNabb Miller, Chairman, Department of Public Welfare of the General Federation of Women's Clubs.
Dr. H. W. Wiley, Honorary President, Association of Official Agricultural Chemists.

These addresses, with the exception of those given by the president and honorary president of this association, W. W. Randall[1] and Harvey W. Wiley[2], respectively, were published by the Association of Dairy, Food and Drug Officials.

On Tuesday afternoon the women were given an auto trip followed by luncheon at the Congressional Country Club; on Tuesday evening all the delegates and guests were invited to an oyster roast at the Corinthian Yacht Club; on Wednesday evening the usual informal dinner in honor of H. W. Wiley was given by the men, and the women were invited to a theater party; on Thursday morning the women were taken to the laboratories of the Department of Agriculture and of the National Canners Association; and on Thursday afternoon all the members and guests took a trip to Mt. Vernon and Alexandria.

ENTERTAINMENT COMMITTEE.

The entertainment committee was composed of the following members:

W. W. Skinner, Chairman, Bureau of Chemistry; Oswald Schreiner and R. B. Deemer, Bureau of Plant Industry; Paul E. Howe, Bureau of Animal Industry; C. Rowena Schmidt, Bureau of Home Economics; W. H. Ross, Bureau of Soils; Ernest Kelly, Bureau of Dairying; and L. H. Almy, G. L. Bidwell, P. B. Dunbar, H. A. Lepper,

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[1] *This Journal*, 1927, **10**: 16.
[2] See page 436.

A. S. Mitchell, J. W. Sale, R. W. Balcom, F. C. Blanck, W. S. Frisbie, F. B. Linton, A. G. Murray, and F. P. Veitch, Bureau of Chemistry.

HOSTESSES.

The following women acted as hostesses:

Mrs. C. A. Browne, Chairman; Mrs. J. S. Abbott, Mrs. W. D. Bigelow, Mrs. P. B. Dunbar, Mrs. D. R. Forbes, Mrs. W. S. Frisbie, Mrs. W. P. Jones, Mrs. H. M. Loomis, Miss M. E. Lapp, Mrs. A. S. Mitchell, Mrs. Junior Owens, Mrs. W. W. Skinner, and Mrs. H. W. Wiley.

The proceedings for Thursday were Published in Vol. X, No. 1.

CONTRIBUTED PAPERS.

THE IODOMETRIC EVALUATION OF METHYLENE BLUE.

By Walter C. Holmes (Color and Farm Waste Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.).

In the method adopted by the Association of Official Agricultural Chemists for the determination of methylene blue in medicinal samples the estimation of the dye is based upon its iodine absorption when precipitated in the presence of acetic acid by the addition of a large excess of iodine solution[1].

From the comment on the method by the associate referee it would be inferred that the employment of acetic acid helps to a great extent in "completing the reaction", i. e., in causing as much iodine as possible to react with the dye. The "completed" reaction is understood by the writer to be the formation of a complex containing five atoms of iodine per molecule of the dye. The implication of the associate referee seems to be that the dye will not absorb a larger proportion of iodine.

Recent investigation[2] of the reactions of several other basic dyes with iodine led the writer to question the validity of these conclusions insofar as they pertain to methylene blue. With the dyes in question the presence of acids was invariably found to restrict rather than promote the absorption of iodine. The quantity of iodine that was taken up by the dye was found to depend, primarily, upon the concentration of iodine in the solution with which the precipitated dye-iodine complex was in equilibrium. Complexes of definite atomic proportions were not obtained.

In view of these facts it appeared advisable to undertake a further investigation of the official iodometric method for methylene blue.

In order to determine the influence of variation in acidity upon the iodine absorption of the dye, a series of determinations was carried out in which all other factors were maintained constant. The general provisions of the test corresponded to those of the official method. The total volume of the solution was 200 cc. in all instances. The dye used was a "medicinal" methylene blue of good quality, the dye content of which was determined by the reduction method with titanous chloride.

The influence of variation in acidity upon the reaction is well defined. The presence of acid does not promote the absorption of iodine; on the contrary, it restricts it. The effect of slight variations in acidity is negligible.

The influence of variation in dye concentration was tested with a second sample of methylene blue which had been carefully evaluated by

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[1] *This Journal*, 1923, 7: 20.
[2] Unpublished.

TABLE 1.

Influence of variation in acidity.

(0.0767 gram of anhydrous dye, 0.7627 gram of iodine added.)

NO.	GLACIAL ACETIC ACID	0.1 *N* IODINE ABSORBED	IODINE ABSORBED PER MOLECULE OF DYE
	cc.	*cc.*	*atoms*
1	. .	13.46	5.60
2		13.42	
3	15	12.88	5.35
4	"	12.78	
5	30	12.13	5.06
6	"	12.18	
7	"	12.15	
8	45	11.65	4.86
9	"	11.67	

the reduction method. The essential provisions of the official method were followed, and all details of manipulation were kept as uniform as possible throughout all the determinations.

TABLE 2.

Influence of variation in dye concentration.

(30 cc of glacial acetic acid and 0.7563 gram of iodine added.)

NO.	ANHYDROUS DYE	0.1 *N* IODINE ABSORBED	IODINE ABSORBED PER MOLECULE OF DYE
	gram	*cc.*	*atoms*
10	0.100	15.35	4.91
11	"	15.34	
12	"	15.35	
13	0.080	12.38	4.95
14	"	12.38	
15	"	12.38	
16	0.060	9.43	5.00
17	"	9.32	
18	"	9.39	
19	0.040	6.40	5.15
20	"	6.46	
21	"	6.46	
22	0.020	3.52	5.50
23	"	3.42	
24	"	3.39	

The reaction involved is not that of the formation of a complex of definite atomic proportions[1]. The proportion of iodine absorbed is variable. It increases with the decrease in dye concentration, owing to the fact that the residual iodine concentration in the solution increases with the decrease in dye concentration. It is the concentration of residual iodine in the solution that determines the iodine absorption. The official method is fundamentally faulty in ignoring the operation of that factor.

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[1] *Bull. Soc. Chim.*, 1909, 4, 5: 626.

For accurate work the official method must be calibrated for variation in dye concentration. The iodine absorption of various quantities of dye must be determined by actual test, as was done in the experiments recorded in Table 2. The data of Table 2, however, are *not* recommended for indiscriminate adoption. The method is one that may be affected by the slightest variation in technique. Any slight change in filtering the solutions, in particular, may cause appreciable variations in results. It is advisable, therefore, for each analyst to callibrate the method for himself.

SUMMARY.

In the precipitation of methylene blue by iodine solutions the presence of acid restricts the absorption of iodine by the dye, and the proportion of iodine taken up is dependent, primarily, upon the concentration of residual iodine in the solution.

For accurate work the official method must be calibrated for variation in dye concentration.

RAPID BOILING AS AN AID TO A SHORTENED PERIOD OF DIGESTION IN THE DETERMINATION OF NITROGEN[1].

By O. M. SHEDD[2] (Kentucky Agricultural Experiment Station, Lexington, Ky.).

HISTORICAL PART.

Several modifications have been suggested and some material improvements have been made in the original method devised by Kjeldahl (7) for determining nitrogen in organic materials. The chief objects in view were to perfect the accuracy of the original method, to widen its scope of application, and to improve its technique by shortening the time required for the digestion.

Heretofore, the most valuable contributions to the method were the introduction of a catalyst, which was probably first suggested by Arnold (1), to accelerate the oxidation, and the addition of a salt such as potassium sulfate, recommended by Gunning (4), to raise the boiling point of the solution. When nitrate is present, the salicylic acid modification devised by Scovell (17) has proved the most satisfactory.

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[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The writer desires to thank Dr. A. M. Peter, Head of the Department of Chemistry, for helpful criticism in the work and in the preparation of the manuscript; also H. D Spears and others in the Feed Control Laboratory for courtesies extended during this investigation.

Arnold and Wedemeyer (2) probably first suggested that the Arnold and Gunning modifications, with some changes, be consolidated with the original Kjeldahl method to constitute what is now known as the Kjeldahl-Gunning-Arnold method—no doubt the method most commonly used at the present time. If nitrate is present, the Scovell modification can be incorporated with any of the procedures previously mentioned.

Although the catalyst generally recommended for the digestion is either mercury or copper, including their compounds, a hasty survey of the literature seems to show that mercury or its oxide is the choice of some investigators, owing to the slightly higher results obtainable. The salt generally recommended to raise the boiling point in the digestion is potassium sulfate, although sodium sulfate—and possibly other compounds—has been found to be satisfactory.

The original Kjeldahl method consisted of the oxidation of the organic nitrogenous material by digestion in concentrated sulfuric acid, at or near its boiling point, the oxidation being completed by the addition of potassium permanganate. This was a tedious process requiring much time to complete the oxidation of some materials and, for this reason, it was not always satisfactory. Later, the use of proper catalysts to accelerate the oxidation, together with the simultaneous addition of a substance to raise the boiling point of the acid solution, materially improved the accuracy of the original procedure. Moreover, these changes have shortened the process and increased its application until, at the present time, there are comparatively few compounds of nitrogen in which this constituent cannot be determined. Nevertheless, a study of the literature on this subject shows that in recent years some investigators have continued work along these lines.

Pickel (14) mentions that the digestion of certain materials by the Kjeldahl-Gunning-Arnold method can be completed in 30 minutes. He analyzed dried blood, calcium cyanamide, cottonseed meal, and miscellaneous feeding stuffs. Most of his comparisons were 30 minute versus 90 minute digestion, with the full or almost the full flame of a Bunsen burner.

Mears and Hussey (11) found that small quantities, not to exceed 2 cc. of perchloric acid (60 per cent), in the sulfuric acid digestion shortened the time required for this operation without affecting the accuracy of the result, the only exception being urine. Larger quantities of perchloric acid usually proved detrimental. They used a variety of materials consisting of milk, urine, casein, tankage, albumen, gelatin, dried blood, sheep manure, coconut shells, castor pumace, rape seed meal, cottonseed meal, and hoof meal. Parker and Terrell (12), also, found that perchloric acid aided in the oxidation of leather. They used 6 cc. of this acid in digesting 8 grams of leather and heated for 1 hour.

Kleeman (8) states that 25 cc. of hydrogen peroxide (30 per cent), added to 1 gram of the sample before the addition of the sulfuric acid in the digestion, materially shortens this operation. He generally found 25 to 30 minutes' heating sufficient but recommended 45 minutes. Heuss (6) finds this chemical effective and advises about the same period of heating as Kleeman. Liljevall (9) also reports that it is an effective supplement to the oxidizing agents. Sborowsky and Sborowsky (16) found that a pinch of mercurous iodide substituted for 1 gram of mercury oxidizes the carbonaceous matter more rapidly. Richards (15) corroborates this in the digestion of leather and coal, using 0.25 gram of mercurous iodide. On the other hand, Hassig (5) reports that in his work the process was not hastened by this compound, but rather that its use was disadvantageous owing to sublimation of the iodide in the neck of the flask.

Parri (13) states that in the digestion of flour the time required to obtain a clear solution is less when a mixture of vanadium pentoxide and cupric oxide is used than when either of these catalysts is used alone. With 1 gram of flour, 20 cc. of sulfuric acid, 0.1 gram of vanadium pentoxide, and 0.5 gram of cupric oxide, the time was 2.2 hours, and with either catalyst separately, the time was 6 hours.

EXPERIMENTAL PART.

The estimation of nitrogen being one of the most common quantitative determinations made in analytical laboratories, it was thought that any satisfactory change of the Kjeldahl-Gunning-Arnold method that would shorten the time required for an analysis would be advantageous.

As previously mentioned, the reason for adding a sulfate is to raise the boiling point of the acid solution so as to complete the oxidation as quickly as possible. Therefore, an attempt was made to determine what effect the application of more intense heat than could be supplied by an ordinary laboratory burner would have on accelerating the digestion in the Kjeldahl-Gunning-Arnold method.

As the manufacturers of the modern grid burners, like the Meker and Fisher, claim that they give more heat than the ordinary Bunsen, it was decided to use a Fisher burner[1] (Laboratory Model type), the selection of the particular make being entirely by chance. Natural gas was used, but it was found important to provide a good flow and pressure, otherwise low results are to be expected in the short digestion. The local gas normally has 1000–1100 B. T. U. per cubic foot, and it is ordinarily maintained at a minimum pressure of approximately 3 ounces. During extremely cold weather with very low gas pressure, somewhat lower results were obtained in a few instances with Burner "b" in the short digestion. These results are not given in the tables because higher figures were obtained on the same samples when the pressure was normal. However, they are the only results obtained during this investigation not reported.

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[1] Hereafter referred to as Burner "b", and the ordinary laboratory or Bunsen burner is referred to as Burner "a".

Because the directions were not at hand, and owing to a lack of experience in the use of Burner "b", the gauze located under the grid was not removed, as recommended when natural gas is to be used. Hotter cones, or at least ones more easily controlled, probably could have been obtained if this had been done. The grid, however, was used and not the cap. A higher temperature would have been attained if the flask had been placed in the heart of the flame, about 0.5 inch above the top of the burner as recommended, but this was not done because it was not desired to heat the flask more than was necessary above the boiling solution in order to avoid a possible decomposition and loss of ammonia.

It is of interest to mention in this connection, following the suggestion of A. M. Peter, that temperature readings of the boiling solution in the digestion made with a quartz thermometer by the writer in this investigation were the same regardless of the type of burner used. Burner "a" and Burner "b" employed elsewhere in this work and also a Tirrill burner were compared. The Tirrill burner is designated as Burner "c".

The boiling-point readings with 25 cc. of sulfuric acid, specific gravity, 1.84; 0.7 gram of mercury; and other additions indicated were as follows:

$Na_2SO_4.H_2O$ *gram*	COTTONSEED MEAL *gram*	BURNER USED	°C.
..	...	"a", "b", "c"	321
18	...	" " "	357
18	0.7	" " "	364
27	...	" " "	367

The solutions boiled in less time and more vigorously over Burner "b" than over the other burners. Moreover the solution containing the cottonseed meal was clear in 10 minutes, and similar solutions boiled over the other burners were not entirely clear after 20 minutes.

The method employed, except where otherwise noted, was the Kjeldahl-Gunning-Arnold with a modification used by H. D. Spears, of the Feed Control Laboratory of this Station, which he has found very satisfactory. He uses cupric sulfate instead of mercury as a catalyst and also adds potassium polysulfide[1] in the distillation because he has found it advantageous for obtaining an even-boiling solution. The writer has found that potassium polysulfide serves another purpose—that of partly retarding the carrying over of sodium hydroxide as a spray in the excessive hydrogen gas generated if a large excess of zinc and sodium hydroxide should be present in the distillation when cupric sulfate is used. This error will occur even when an effective trap is used. As an illustration of such losses as compared with the usual excess of these chemicals, distillations were made in the same manner and with the same apparatus that was used in the method described later, except for the quantity of reagents added. The results obtained are given in Table 7. Of course, the excess of sodium hydroxide present in some of the distillations was abnormal, but it is of interest to note that practically none was found in the distillate when the cupric sulfate and zinc were absent. It appears that when copper is in solution there is more of a tendency to carry over sodium hydroxide because in every instance when it was precipitated by potassium polysulfide, less sodium hydroxide was found in the distillate. Similar results would probably be obtained when soluble mercury instead of copper was present. The results in Table 7 show that in the distillation it is best to use a minimum quantity of sodium hydroxide but sufficient to liberate the ammonia and a minimum quantity of finely granulated zinc (100 mmg. or less—just sufficient to prevent bumping), and to precipitate copper or mercury with potassium polysulfide.

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[1] The "potassium sulfide, fused" of the dealers. Used in 4 per cent solution.

The method found most satisfactory for the short digestion and used in this work unless otherwise noted is as follows:

METHOD.

A sample of 0.7005 gram[1] was put into the round-bottom Pyrex flask[2] that was used for the distillation, and 18 grams of sodium sulfate (dry) was added and mixed thoroughly with the sample. To this mixture was added 25 cc. of sulfuric acid (sp. gr. 1.84) and 0.7 gram of mercury[3]. The contents were mixed, and the flask—with the bottom about 2½ inches from the grid—was heated over the Fisher burner for 20 minutes with an approximately 6 inch flame. The solution was clear and had a pale straw color in 10 minutes or less. It is best to continue the heating for at least 10 minutes after clearing[4]. The contents were cooled, 300 cc. of distilled water was added, then a mixture containing 70 cc. of sodium hydroxide (63 per cent) and 25 cc. of potassium polysulfide solution (4 per cent)[5], and finally not over 0.1 gram of zinc (granulated). The distillation was made over the free flame of a Bunsen burner, a block-tin tube condenser fitted with a trap (10) being used, and distillation was usually continued until the solution in the flask commenced to bump. The distillate of about 200 cc. was collected in an excess of standard sulfuric acid containing 4 drops of alizarin sodium sulfonate (1 per cent aqueous solution) and titrated with 0.1 *N* sodium hydroxide. Blank determinations were made on the reagents and deducted.

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[1] In the samples of nicotine, soils, and milk, approximately 0.5 gram, 7 grams, and 10 grams were used, respectively.

[2] Capacity, 800 cc., diameter and length of neck, 1 inch and 7¾ inches, respectively.

[3] When $CuSO_4.5H_2O$ was used as the catalyst, 0.25 gram was added, unless otherwise noted.

[4] Digestions which gave good results were made in 15 minutes. In order to determine if H_2O in the material would prolong the time, a sample of cottonseed meal, No. 73104, had 10 cc. distilled H_2O added, after which the digestion was made in 20 minutes. The result was 42.25 per cent protein. The average of four determinations by the same method without the H_2O present was 42.26 per cent protein as given in Table 2.

[5] When $CuSO_4$ $5H_2O$ alone was used, 10 cc. of the potassium polysulfide solution was added.

Using this method, the writer has made nitrogen determinations in 1 hour. Digestions were also made with cupric sulfate and with this catalyst plus mercury. The 5 hour digestions were also made by the same method except that the ordinary laboratory burner was used.

Comparisons were made of the short and regular 5 hour digestions on a variety of feedstuffs, fertilizers, soils and other materials. The mixed feeds used in this work included wheat (bran, middlings, screenings, shipstuff), corn (bran, gluten, meal products), Milo maize, oats (meal, products), barley, rye, buckwheat, rice bran, alfalfa meal, coconut oil meal, cottonseed meal, sunflower seed, linseed oil meal, brewers' dried grains, molasses, tankage, meat scrap and weed seeds.

The results on the samples listed and on other samples employed in this work are given in Tables 1 to 7.

TABLE 1.

Comparison of amounts of protein in mixed feeds[*] determined by short digestion with amounts found after 3 hours' digestion in which different catalysts were used.

SAMPLE NUMBER	SHORT DIGESTION		3 HOUR DIGESTION[†]
	Burner "b"		Burner "a"
	$CuSO_4.5H_2O$	Hg	$CuSO_4.5H_2O$
	per cent	*per cent*	*per cent*
73328	11.13	11.38	10.94
			10.88
Average			10.91
73331		15.06	14.50
73332	17.44	17.81	17.56
73334		10.69	10.44
73336		11.19	11.00
73338	17.25	17.25	17.38
73339		11.88	11.75
73349	21.63[‡]	21.94	22.00
		22.00	
Average		21.97	
73353	21.00[‡]	21.19	21.25
73357	23.88[‡]	24.00	23.88
73359	24.19[‡]	24.44	24.38
73471	17.88[‡]	18.25	18.00

[*] The term "mixed feed" used here signifies a mixture of two or more different ingredients and should not be confused with similar classification by feed control laboratories.

[†] Determinations by the 3 hour digestion were made by the Feed Control Laboratory.

[‡] Used 2.75 grams of $CuSO_4.5H_2O$, i. e. 0.70 gram of Cu.

DISCUSSION AND CONCLUSIONS.

The results obtained on the samples used in this work show that with the exception of pyridine a satisfactory digestion in the Kjeldahl-Gunning-Arnold method can be made in a short period of time, not exceeding

TABLE 2.

Comparison of amounts of protein in cottonseed meals determined by short digestion over different burners with amounts found after 5 hours' digestion in which different catalysts were used.

SAMPLE NUMBER	SHORT DIGESTION				5 HOUR DIGESTION		
	Burner "a"	Burner "b"			Burner "a"		
	$CuSO_4 \cdot 5H_2O$*	$CuSO_4 \cdot 5H_2O$*	Hg†	$CuSO_4 \cdot 5H_2O$+Hg‡	$CuSO_4 \cdot 5H_2O$	Hg	$CuSO_4 \cdot 5H_2O$+Hg
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
70936	41.97	41.69	42.63 42.56	42.44	42.75	42.25 42.56	
Average			42.60			42.41	
70942	39.31	39.69	39.44 39.50 39.75	40.12	39.94	39.63 39.50	
Average			39.56			39.57	
73039	34.13	33.19	34.56	34.44	34.44	33.88 34.25 34.25	
Average						34.13	
73081	39.50	39.62	39.94	39.94	40.13	39.63	
73104		42.25 41.94 42.25 42.19 42.06 41.63 42.56	42.63 42.63 41.88 41.88	42.44 42.44 41.81 42.13 42.38 42.56 42.69 42.25	42.88 42.63 42.50 42.31 42.50	41.94 42.13	42.06 42.31
Average		42.13	42.26	42.34	42.56	42.04	42.19
73404			39.88 40.13		40.19	40.13	
Average			40.01				
73481			39.44			39.25 39.31	
Average						39.28	
General average of 70936 70942 73039 73081	38.72	38.55	39.17	39.24	39.32	38.94	
General average of 70936 70942 73039 73081 73104		39.26	39.78	39.86	39.96	39.56	
General average of all samples			39.77			39.60	

* 0.25 gram $CuSO_4 \cdot 5H_2O$ and heated 30 minutes.
† 0.70 gram Hg and heated 20 minutes.
‡ 0.25 gram $CuSO_4 \cdot 5H_2O$ + 0.70 gram Hg, and heated 30 minutes.
¶ The general average is not given in every instance because all determinations were not made on each sample.

TABLE 3.

Comparison of amounts of protein in tankages determined by short digestion over different burners with amounts found after 5 hours' digestion in which different catalysts were used.

SAMPLE NUMBER	SHORT DIGESTION				5 HOUR DIGESTION	
	Burner "a"	Burner "b"			Burner "a"	
	$CuSO_4 \cdot 5H_2O$*	$CuSO_4 \cdot 5H_2O$*	Hg†	$CuSO_4.5H_2O + Hg$‡	$CuSO_4 \cdot 5H_2O$	Hg
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
70939	55.63	56.63	56.50 56.44	56.94	56.88	56.31 56.50
Average			56.47			56.41
72837	56.13	56.12	58.38 58.31	57.88 57.69 57.81	57.13	58.25 58.00 58.00
Average			58.35	57.79		58.08
72843	58.13	58.62	58.69 58.38 58.13	59.06	59.56	58.50 58.75 58.75
Average			58.40			58.67
73083	60.00	59.32	61.50	61.50	61.25	61.26 61.56
Average						61.41
General average	57.47	57.67	58.68	58.82	58.71	58.64

* 0.25 gram $CuSO_4.5H_2O$ and heated 30 minutes.
† 0.70 gram Hg and heated 20 minutes.
‡ 0.25 gram $CuSO_4.5H_2O$ + 0.70 gram Hg, and heated 30 minutes.

20 minutes by the method presented, provided mercury, not copper, is used as the catalyst. A combination of mercury and copper did not have any advantage over mercury alone.

It is of interest to note that neither Burner "a" nor Burner "b" used in the short digestion with cupric sulfate as the catalyst was satisfactory in the few instances where comparisons were made, as shown in Tables 2 and 3. Nevertheless, it will be seen in the tables, with one exception mentioned later, that Burner "b" with mercury as the catalyst in the short digestion gave results which were comparable with those found by the official method[1]. This is of importance inasmuch as the use of mercury or copper as catalysts is optional in the present official Kjeldahl-Gunning-Arnold method (3). However, as the tables show, the results

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[1] Burner "a" with mercury as the catalyst was not used in the short digestion. H. D. Spears informed the writer that he had used it with this catalyst in 30 minute digestions but did not always find it satisfactory.

TABLE 4.

Comparison of amounts of protein in miscellaneous feeds and other materials determined by short digestion with amounts found after 5 hours' digestion in which different catalysts were used.

SAMPLE NUMBER	CHARACTER OF SAMPLE	SHORT DIGESTION BURNER "b"			5 HOUR DIGESTION BURNER "a"		
		$CuSO_4 \cdot 5H_2O$	Hg	$CuSO_4 \cdot 5H_2O + Hg$	$CuSO_4 \cdot 5H_2O$	Hg	$CuSO_4 \cdot 5H_2O + Hg$
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
73343	Meat scraps		53.31		53.76*		
73345	Corn gluten meal	40.69	41.31		41.50*		
73346	Wheat products	15.19†	15.25		15.13* 15.13*		
Average					15.13*		
73350‡	Linseed oil meal	30.75‡	31.38		31.13*		
73410	Rice bran	12.56†	12.75		12.75*		
73426	Wheat products	16.13‡	16.50 16.13		16.25*		
Average			16.32				
80826	Nicotine			16.08¶ 16.26¶			16.19¶ 16.19¶
Average				16.17¶			16.19¶
86524	Peanut oil meal		43.88 44.19			44.31	
Average			44.04				
86525	Peanut shucks		5.56			5.69	
86526	Coconut meal		23.25			23.25	
86527	Soluble blood flour		89.38 88.56			88.63 89.13	
Average			88.97			88.88	
86528	Blood meal		86.44 86.06			86.88	
Average			86.25				
86529	Beef meal		39.88 40.00			40.31 40.00	
Average			39.94			40.16	

---

* Determinations made by Feed Control Laboratory according to usual routine in which low protein feeds are digested for at least 3 hours and high protein feeds for at least 4 hours.

† Used 0.75 gram of $CuSO_4.5H_2O$

‡ Used 2.75 grams of $CuSO_4$ $5H_2O$, i e 0.70 gram Cu.

¶ Nitrogen. This sample contained 93.86 per cent of nicotine, i e. 16.21 per cent of nitrogen. In the determination where 16.26 per cent nitrogen was obtained, 1 gram of cane sugar was used in the digestion

§ All results on pyridine represent nitrogen. Theory, 17.72 per cent nitrogen This sample although labeled "pure" probably did not contain the theoretical amount of nitrogen.

** 1 gram of cane sugar used in the digestion.

†† 2.75 grams of $CuSO_4.5H_2O$ and 1 gram of cane sugar used in the digestion.

‡‡ Determinations made by H. D. Spears, and the digestions were for 3 hours.

¶¶ Determinations made by H. D. Spears, and digestions were for 5 hours.

§§ 2.75 grams of $CuSO_4$ $5H_2O$, 0.70 gram of Hg, and 1 gram of cane sugar used in the digestion.

TABLE 4.—Continued.

SAMPLE NUMBER	CHARACTER OF SAMPLE	SHORT DIGESTION BURNER "b"			5 HOUR DIGESTION BURNER "a"		
		$CuSO_4.5H_2O$	Hg	$CuSO_4.5H_2O + Hg$	$CuSO_4.5H_2O$	Hg	$CuSO_4.5H_2O + Hg$
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
86530	Beef scraps		65.25			65.63	
86531	Poultry bone meal		26.44			26.19	
86532	Dried buttermilk		30.00			29.81	
86533	Skim milk		3.76			3.76	
			3.76			3.76	
Average			3.76			3.76	
86535	Casein (Hammerstein)		88.04		88.49		88.49
			88.04		88.55		
			88.04		88.52		
86607	Pyridine§	6.25	11.42	9.78	12.76	16.98	16.99
		4.84‡	8.21	16.92	12.24‡	17.12	17.11
		6.41**	13.72	16.78	13.70††	17.16	17.21
		5.60††	16.76**	8.72	7.48‡‡	16.98‡‡	17.16
				8.55	11.73¶¶	17.04¶¶	17.16**
				.04			17.10§§
				none			
				10.50			
				17.03**			
				11.44**			
				14.08**			
				17.14**			
				15.08**			
				17.14**			
				0.74**			
				14.31**			
				16.77**			
				12.98**			
				16.44**			
				17.03**			
				17.10**			
				12.66**			
				14.46**			
				10.92§§			
Average		5.78	12.53	12.36	11.58	17.06	17.12

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* Determinations made by Feed Control Laboratory according to usual routine in which low protein feeds are digested for at least 3 hours and high protein feeds for at least 4 hours.

† Used 0.75 gram of $CuSO_4.5H_2O$.

‡ Used 2.75 grams of $CuSO_4.5H_2O$, i. e. 0.70 gram Cu.

¶ Nitrogen. This sample contained 93.86 per cent of nicotine, i. e. 16.21 per cent of nitrogen. In the determination where 16.26 per cent nitrogen was obtained, 1 gram of cane sugar was used in the digestion

§ All results on pyridine represent nitrogen. Theory, 17.72 per cent nitrogen. This sample although labeled "pure" probably did not contain the theoretical amount of nitrogen.

** 1 gram of cane sugar used in the digestion.

†† 2.75 grams of $CuSO_4.5H_2O$ and 1 gram of cane sugar used in the digestion.

‡‡ Determinations made by H. D. Spears, and the digestions were for 3 hours.

¶¶ Determinations made by H. D. Spears, and digestions were for 5 hours.

§§ 2.70 grams of $CuSO_4.5H_2O$, 0.70 gram of Hg, and 1 gram of cane sugar used in the digestion.

TABLE 5.

Comparison of amounts of nitrogen in fertilizer determined by short digestion with amounts found after 5 hours' digestion in which different catalysts were used.

SAMPLE NUMBER	CHARACTER OF SAMPLE	SHORT DIGESTION BURNER "b"		5 HOUR DIGESTION BURNER "a"	
		$CuSO_4.5H_2O$	Hg	$CuSO_4$ $5H_2O$	Hg
		per cent	*per cent*	*per cent*	*per cent*
63595	Complete fertilizer		1.77		1.71*
63601	Complete fertilizer		0.97		1.00*
63605	Complete fertilizer		0.99		0.98*
63607	Complete fertilizer		1.88		1.86*
63619	Complete fertilizer		0.88		0.91*
63621	Complete fertilizer		1.70		1.74*
63899	Complete fertilizer		5.92		5.95
			5.91		5.83
			5.89		
Average			5.91		5.89
64773	Pure bone meal		3.17		3.24
			3.23		3.24
			3.19		3.28
Average			3.20		3.25
64776	Complete fertilizer		1.23		1.22*
64777	Complete fertilizer		1.69		1.74*
64813	Complete fertilizer		2.08		1.97
64828	Pulverized manure	16.45	16.68	16.68	16.64
		16.60	16.67	16.56	16.54
			16.68		
			16.68		
Average		16.53	16.68	16.62	16.59

* Determinations made by the Fertilizer Control Laboratory according to usual routine in which the digestions were made for at least 3 hours.

with cupric sulfate were about as good as with mercury in the 5 hour digestion, the only exception in favor of the mercury being with pyridine.

The samples employed in this investigation comprised various kinds of feeds, fertilizers, soils, and other miscellaneous materials. Accurate nitrogen determinations by the short digestion were completed in 1 hour, although the average time was probably 1¼ hours, depending on the gas pressure.

Copper did not always prove as effective as mercury as a catalyst in the short digestion, although it was satisfactory in the official method, except with pyridine. No benefit was derived from the use of a quantity of copper equivalent to the mercury. Frequently, good results were procured with copper in the short digestion, but oftentimes, and without any apparent reason, low results were obtained with it on similar samples under the same conditions.

Pyridine was the only sample in which the nitrogen could not consistently be determined with mercury as a catalyst in the short digestion,

TABLE 6.

Comparison of amounts of nitrogen in soils determined by short digestion with amounts found after 5 hours' digestion in which mercury was used as the catalyst.

SAMPLE NUMBER	SHORT DIGESTION BURNER "b"	5 HOUR DIGESTION BURNER "a"
	per cent	*per cent*
14411	0.313	0.318
25796	0.138	0.140
36263	0.076	0.076
36691	0.085	0.088
50737	0.190	0.197
56493	0.220	0.229
61999	0.142	0.146
	0.142	
Average	0.142	
80137	0.118	0.124
80440	0.219	0.224
	0.215	
Average	0.217	
80874	0.188	0.189
	0.187	
Average	0.188	
81124	0.168	0.174
	0.172	
Average	0.170	
81237	0.112	0.112
	0.110	
Average	0.111	
81250	0.167	0.172
	0.166	
Average	0.167	
General average	0.164	0.168

notwithstanding it was tried under various conditions, as shown in Table 4. Copper also failed as a catalyst with this substance both by the short and by the regular digestion of the official method. Considerably higher and more consistent results, which agreed more closely with the theoretical, were obtained with mercury than with copper on pyridine by the long digestion. In the determinations in which low results were obtained, the odor of pyridine was distinct in the distillate, showing that the reaction was not complete in the digestion. No odor of pyridine, however, was apparent in the distillates from the long digestion with mercury.

The best conditions advised for the distillation are (1) the use of a

TABLE 7.

Effect of excessive amounts of NaOH and Zn in carrying over alkali in the distillate when $CuSO_4$ $5H_2O$ *was employed as the catalyst.*

NaOH	POTASSIUM POLYSULFIDE	$CuSO_4$ $5H_2O$	Zn	DISTILLED H_2O	0.1 *N* HCl REQUIRED TO NEUTRALIZE THE DISTILLATE
cc.	*cc.*	*gram*	*gram*	*cc.*	*cc.*
150	none	0.25	3.00	300	5.65
150	10	0.25	3.00	300	4.59
150	10	0.25	3.00	300	4.51
150	none	0.25	0.25	300	0.90
150	none	0.25	0.25	300	0.90
150	10	0.25	0.25	300	0.13
150	none	0.25	0.10	300	0.16
150	10	0.25	0.10	300	0.13
150	none	none	0.25	300	0.60
150	none	none	0.25	300	0.60
150	none	none	none	300	0.03
90	none	0.25	0.10	300	0.13
90	10	0.25	0.10	300	0.08
10	none	0.25	3.00	300	0.10
10	10	0.25	3.00	300	0.08
10	none	0.25	0.10	300	0.05
10	10	0.25	0.10	300	0.03

minimum quantity of sodium hydroxide, but sufficient for the liberation of the ammonia; (2) a minimum quantity of zinc, not over 100 mmg., to prevent bumping; and (3) precipitation of the copper or mercury with potassium polysulfide.

It may be concluded that the short digestion can be satisfactorily applied to samples of a character different from those used in this work, although it is recognized that there are undoubtedly some synthetic compounds containing nitrogen, and possibly other substances, in which this constituent cannot be determined by the above procedure, just as there are some in which it cannot be obtained in a long digestion by any of the improved methods now in use.

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NOTE ON THE DETERMINATION OF TOTAL SOLIDS IN MALT VINEGAR.

By JOHN F. LAUDIG (The H. J. Heinz Company Laboratories, Pittsburgh, Pa.).

It has been the experience of the writer that the official method of the A. O. A. C. for the determination of solids in vinegar[1] gives excellent and concordant results when applied to cider vinegar. Woodman[2], however, considered a modification necessary when this method was used for the determination of solids in cider vinegar, owing to the persistent retention of acetic acid in the solids, and found that more accurate results were obtained by adding 5 cc. of water to the residue and again evaporating to dryness, making three evaporations in all before cooling and weighing.

In control work on malt vinegar the writer was unable to obtain wholly consistent results with the official method. Although check analyses agreed closely, variations as high as 0.8 per cent were encountered when determinations were made on the same sample upon different days. The acidity of the solids found fluctuated directly as the solids. Thus the difficulty appeared to be the result of the retention of acetic acid by the malt vinegar solids in amounts varying according to the daily fluctuations in relative humidity and atmospheric pressure.

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[1] *Methods of Analysis, A. O. A. C.*, 1925, 325.

[2] Food Analysis, 2nd ed., 1924, p. 374.

To overcome this influence of weather conditions, a modification of the A. O. A. C. method was used, and lower but comparable results were obtained. The modified method is as follows:

A 10 cc. sample is measured into a tared, flat-bottomed platinum dish of 50 mm. bottom diameter, evaporated on a boiling water bath for 20 minutes, taken up in 5–8 cc. of distilled water, again evaporated and taken up with 5–8 cc. of distilled water, and finally evaporated to dryness (30 minutes), dried for exactly 2.5 hours in a water oven at the temperature of boiling water, cooled in a desiccator, and weighed.

A comparison of the results obtained with cider vinegar and malt vinegar by the official and modified methods is shown in Table 1.

TABLE 1.

Results of determination of solids in cider and malt vinegars by the official and modified methods.

DATE	CIDER VINEGAR		DATE	MALT VINEGAR			
	Official Method	Modified Method		Sample No. 65		Sample No. 66	
				Official Method	Modified Method	Official Method	Modified Method
	per cent	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
8- 9-27	1.44	1.40	3-7-27	2.22	2.17	2.12	1.99
	1.41	1.40		....	....	....	....
8-10-27	1.40	1.39	4-4-27	3.03	2.17	2.17	2.00
	1.44	1.39		2.87	2.19	2.21	1.96
8-11-27	1.39	1.40	4-5-27	2.95	2.17	2.15	2.01
	1.41	1.38		2.87	2.15	2.18	1.98
8-12-27	1.43	1.38	4-6-27	3.00	2.19	2.24	2.00
	1.41	1.38		3.02	2.14	2.30	2.02
			4-7-27	2.78	2.13	2.44	1.98
				2.76	2.18	2.41	2.00
			4-8-27	2.63	2.13	2.41	2.01
				2.65	2.14	2.38	2.02
High	1.44	1.40		3.03	2.19	2.44	2.02
Low	1.39	1.38		2.22	2.13	2.12	1.96
Variation	0.05	0.02		0.81	0.06	0.32	0.06

The modified method gave lower results in all cases. While the differences with the cider vinegar are negligible, those with the malt vinegar are too great to be ignored. It would seem that some such modification of the official method would be desirable to make it more accurate and more generally applicable.

The writer wishes to express his appreciation for the helpful suggestions and assistance of L. H. Almy.

THE SPECTROPHOTOMETRIC DETECTION OF BORON.

By WALTER C. HOLMES (Color and Farm Waste Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.).

The turmeric paper test for boric acid is stated by Leach[1] to be delicate to 1 part in 8000. The Henz modification, in which an alcoholic solution of turmeric is employed, is much more sensitive. It is claimed that "if as much as 0.02 mgm. of B_2O_3 is present the residue is colored a distinct reddish brown, while 0.002 mgm. suffices to cause a visible reaction".[2]

Even greater delicacy is attainable by the resort to spectrophotometric methods[3]. The principal absorption of turmeric is in the violet end of the spectrum, in which accurate measurements of light absorption are difficult. One slope of the absorption band extends into the blue region, however, wherein (between 495 and 510 m μ) extinction coefficients may be determined with considerable precision. The compound formed between turmeric and boron is deeper (redder) in color than is turmeric itself. Its absorption band lies nearer the red end of the spectrum, and its absorption in the blue region is proportionately greater. The conversion of turmeric into the turmeric-boron compound, accordingly, results in increased absorption in that region. Conversely, the demonstration of increased absorption therein, under suitable conditions, may be utilized for the detection of boron.

The following procedure is recommended:

A turmeric solution is prepared by extracting turmeric with 95 per cent alcohol and diluting with the same solvent to a point at which 100 cc. of solution contains from 50 to 60 mgm. of total solids; 1 cc. of the aqueous solution to be tested is transferred to a porcelain dish and acidified with 1 cc. of glacial acetic acid, and 2 cc. of the stock turmeric solution is added. The combined solutions are dried on the steam bath, and the residue is dissolved in 5 cc. of glacial acetic acid. A blank test is carried out at the same time in precisely the same manner, 1 cc. of distilled water being used. The extinction coefficients of the final acetic acid solutions are determined in 2 cm. layers over the spectral range between 495 and 510 m μ. If boron is present in the solution under examination, the extinction coefficients obtained in its test will exceed those obtained in the blank test.

The following results were obtained in testing the method with very dilute solutions of boric acid and of borax.

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[1] Food Inspection and Analysis, 4th ed., p. 885.
[2] Treadwell-Hall, Analytical Chemistry, 4th ed., p. 359.
[3] U. S. Bur. Standards Sci. Paper No. 440.

Solution Tested	E at 495 m μ	E at 500 m μ	E at 505 m μ	E at 510 m μ
Blank Test	1.02 0.99 1.00	0.65 0.63 0.64	0.40 0.39 0.40	0.275 0.275 0.275
	Ave. 1.00	Ave. 0.64	Ave. 0.40	Ave. 0.275
Approximately 0.5 parts of boric acid per million parts of water	1.05 1.06 1.06	0.69 0.69 0.68	0.445 0.45 0.455	0.31 0.315 0.305
	Ave. 1.06	Ave. 0.69	Ave. 0.45	Ave. 0.31
Approximately 0.5 parts of borax per million parts of water	1.04 1.07 1.06	0.695 0.715 0.70	0.045 0.045 0.044	0.31 0.30 0.325
	Ave. 1.06	Ave. 0.70	Ave. 0.45	Ave. 0.31
Approximately 1 part of boric acid per million parts of water	1.11 1.10 1.12	0.735 0.73 0.75	0.048 0.0465 0.049	0.36 0.355 0.355
	Ave. 1.11	Ave. 0.74	Ave. 0.48	Ave. 0.36
Approximately 1 part of borax per million parts of water	1.13 1.13 1.15	0.755 0.765 0.77	0.49 0.49 0.50	0.355 0.345 0.355
	Ave. 1.14	Ave. 0.76	Ave. 0.49	Ave. 0.35

A Hilger wave-length spectrometer, equipped with a Nutting photometer, was employed in carrying out the measurements, but any spectrophotometer would prove suitable.

The approximate boron content in the solutions tested ranged between one part in six million and one part in eighteen million. The conclusion appears warranted that if duplicate or triplicate determinations are made with care the test is adequate for the detection of one part of boron in twenty-five million parts of aqueous solution.

NOTE ON THE ASSAY OF SULFONAL TABLETS[1].

By L. E. WARREN (Food, Drug and Insecticide Administration, Washington, D. C.).

A pharmaceutical manufacturer reported that he had experienced some difficulty in the assay of sulfonal tablets and inquired whether the Drug Research Unit could furnish a satisfactory method of assay.

In a moderately complete examination of the literature nothing was found concerning the analysis of sulfonal in tablets. In 1906 Thoms[2] observed that sulfonal might be removed from an aqueous solution almost quantitatively by shaking with chloroform.

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[1] Read before the Division of Medicinal Products, American Chemical Society, at Detroit, Mich., Sept. 7, 1927. Published through courtesy of *Industrial and Engineering Chemistry*

[2] *Ber. deut. pharm. Ges.*, 1896, 6· 283.

The solubility of sulfonal in various solvents was determined by Falck[1], who found that chloroform was the best solvent. Falck also observed that sulfonal begins to sublime at 66°C. and that it is volatile with steam. He suggested that these hitherto unnoted properties might account for the unsatisfactory analytical results previously reported. Falck found that sulfonal might be completely extracted from aqueous solutions by means of chloroform and that 90 per cent could be recovered from urine by extraction with the same solvent.

A search for a tentative method for the assay of sulfonal in tablets indicated that chloroform would be the most satisfactory solvent. It seemed obvious also that heat could not be applied in the evaporation of the solvent or in drying the residue. Several methods were tried. In one a weighed quantity of sulfonal (about 0.5 gram) was mixed with the same quantity of starch and the material was macerated in a beaker for a few minutes with 10 cc. of chloroform. The mixture was transferred to a small filter by means of several small portions of chloroform, and the extraction on the filter was completed by the addition of more of the solvent. The filtrate was evaporated at room temperature by means of a current of air; the residue was dried over sulfuric acid and weighed. The same method was applied to commercial tablets of sulfonal after the material had been pulverized. In other tests the powdered material was extracted with chloroform in a Bailey extractor and the solvent evaporated with the precautions above noted, or the powdered material was placed on the surface of the chloroform layer in a Type C extractor[2], some water was added, and the mixture was extracted with chloroform until exhausted of sulfonal.

Palkin and Watkins[3] have elaborated a device (Type S) for the extraction of powdered materials by upward displacement. This apparatus could undoubtedly be used for the extraction of sulfonal tablets, but it was not tried because the Bailey extractor, a much simpler mechanism, was found to serve well.

The results by the several methods tried are given in Table 1.

COMMENT.

The method using a Type C extractor is time-consuming. The excipient has a tendency to escape into the receiving flask, thus rendering filtration of the chloroform extract compulsory. This method was, therefore, abandoned. Maceration with chloroform, with subsequent percolation on a filter, is also time-consuming. In the absence of extraction apparatus the method would prove serviceable. Extraction in a Bailey

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[1] *Pharm. Zentralhalle*, 1919, 60: 409.
[2] Palkin, Murray, and Watkins, *Ind. Eng. Chem.*, 1925, 17: 612.
[3] *Ind. Eng. Chem.*, 1927, 19: 535.

TABLE 1.

Results of extraction of sulfonal from mixtures.

METHOD	Laboratory Specimen	SULFONAL EXTRACTED FROM—			
		Sample II		Sample III	
		Material taken	Claim	Material taken	Claim
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Maceration and Extraction	101.4	77.0	94.2	86.0	96.3
		77.9	95.3	85.7	95.9
Bailey Extractor	101.3	77.8	95.2	85.5	95.8
	100.8	77.3	94.6	85.9	96.2
				85.3	95.6
Type C Extractor		77.7	95.0		
	100.9	77.7	95.0	85.8	96.0

extractor with chloroform is the most expeditious, the complete exhaustion of a half-gram sample usually requiring not more than an hour. Undoubtedly a Soxhlet extractor would do as well.

After consideration of the results obtained in these three brief studies, the following procedure is suggested for the assay of sulfonal tablets:

ASSAY OF SULFONAL TABLETS.

Weigh 10 tablets individually; ascertain the average weight and the individual variations. Pulverize the tablets and pass the powder through a No. 60 sieve.

Method I.

Weigh a sufficient quantity of the powder to represent at least 5 grains of sulfonal. Macerate the powder in a small beaker with 10 cc. of chloroform and decant the solvent through a small filter. Repeat the extraction with chloroform until the powder is exhausted of sulfonal. Wash the filter with a few cc. of fresh chloroform and evaporate the united solvent in a tared dish at ordinary temperature by the aid of a gentle current of air. Dry the residue to constant weight in a desiccator over sulfuric acid.

Method II.

Weigh a sufficient quantity of the powder to represent at least 5 grains of sulfonal and extract it with chloroform in a Bailey or a Soxhlet extractor until completely exhausted of sulfonal. Evaporate the solvent in a tared dish at ordinary temperature by the aid of a gentle current of air. Dry the residue to constant weight in a desiccator over sulfuric acid.

Each of these methods presupposes that chloroform-soluble substances other than sulfonal are absent from the tablet material.

In order to test the methods further, several manufacturers of sulfonal tablets were asked to assay material from one lot of tablets (Sample II) by each of the two methods. The results reported are given in Table 2, the values found by the writer being included for comparison.

TABLE 2.

Results of assay of sulfonal tablets by methods submitted.

INVESTIGATOR	SULFONAL EXTRACTED BY—			
	Method I		Method II	
	Material taken	Claim	Material taken	Claim
	per cent	*per cent*	*per cent*	*per cent*
R. I. Grantham (Sharp & Dohme)	77.8	95.1	77.6	94.9
G. A. Slothower (H. K. Mulford Co.)	79.8 78.0 77.7 78.1	95.6 93.5 93.2 94.7	78.0* 73.3*	93.5 93.8
C. A. Mikulus (John T. Milliken Co.)	77.1 77.4 77.2	94.3 94.7 94.4	78.4 78.7	95.9 96.3
L. E. Warren	77.0 77.9	94.2 95.3	77.8 77.3	95.2 94.6

* Extracted with ether in a Soxhlet apparatus.

CONCLUSIONS.

Three methods for the assay of sulfonal tablets were tried. Each depended on extraction of the tablet material with chloroform and evaporation of the solvent without heat. In one instance ether was used as the solvent. Extended trials were not carried out, but in the tests made the results were good. Each method gave results of about equal accuracy, but one of the methods is not recommended because of greater difficulties in technique.

The writer wishes to acknowledge his appreciation of the aid received in this brief study from the several pharmaceutical manufacturers and from others who either contributed material or did collaborative work.

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